THE GIANT MYELINATED NERVE FIBRES OF THE PRAWN

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[Plates 22–24]

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Evidence is given that the median and lateral longitudinal giant myelinated fibres in the central nervous system of the prawn *Leander serratus* are syncitial structures, each formed by the fusion of the processes of many segmental nerve cells.

Septa are found at intervals in the axoplasm of the median fibres, but they never completely transect it. They are probably relics of a condition similar to that in the earthworm where the giant fibre running the length of the cord is formed of a chain of segmental syncitial axons each divided from its neighbour by a complete septum which presumably functions as a synapse.

The motor giant fibres, which are segmental and pass out of the central nervous system to the muscles, are the processes of single cells: the axoplasms of the two fibres of the pair in each segment undergo complete fusion with each other and then redivision before leaving the central nervous system.

These motor giant fibres are non-myelinated within the central nervous system, although as great in diameter as other heavily myelinated fibres. They are myelinated outside the central nervous system. In the prawn therefore myelin sheath thickness is not an invariable function of axon diameter.

The lateral giant-fibre synapses show complete axoplasmic discontinuity and their structure does not support Johnson's creation of a new category of synaptic relations.

Two types of synapses between fibres are described. In the first, found in the lateral giant-fibre chain, two myelinated fibres lie closely side by side for a considerable distance, but their neuroplasms are separated by a myelin layer except over an extent of less than 10μ. In the second type, found at the point of contact of both the median and lateral fibres with the motor fibres, a myelinated fibre has synaptic connexions with a large non-myelinated fibre through many fine axonic processes which pass out through a small gap in the myelin sheath.

Introduction

Nerve fibres with a thick myelin sheath are found only rarely in invertebrates, but the fibres of prawns and a few other Crustacea are exceptional, for they are almost as heavily myelinated as vertebrate axons of the same diameter: they are also provided with nodes of Ranvier (Friedländer 1889; Retzius 1890; Nageotte 1916). Some of the prawn myelinated fibres are much larger than any found in vertebrates, for the giant fibres in the central

nervous system may have a total diameter of 60μ (figure 1, plate 22), and in the peripheral nerves the motor fibres reach 90μ or more (figure 3, plate 22). The conduction velocity of fibres of a mean total diameter of about 35μ has been measured, and found to be of the order of 20 m./sec., a value greater than any recorded for fibres of a corresponding size in other Crustacea, but markedly less than that of the largest fibres of even the cold-blooded vertebrates (Holmes, Pumphrey & Young 1941).

The size of the largest prawn fibres makes them unusually convenient material for the study of the structure of the sheaths and synapses. Johnson (1924) claimed to have established the existence of a new type of synapse in the central nervous system of a prawn. At the point of contact of two of the giant fibres he stated that the two axons concerned were partially separated from each other by a demonstrable membrane, but also partially fused, so that over some of their area of contact the axoplasms were continuous. He suggested that this showed that the relationship between neurons could be of a form which was neither true fusion nor synapsis, but intermediate between the two. But it is generally held that if the neuroplasms of two neurons are continuous, then a nerve impulse set up in one of them is transmitted without interruption to the other; while in synapsis the neuroplasms are separate throughout the area of contact, and an impulse set up in one is only transmitted to the other through the synaptic transmission mechanism. It is thus difficult to understand the functional significance of the condition Johnson describes, intermediate between fusion and synapsis, and I have re-examined the prawn giant-fibre synapses histologically in the hope of throwing light on this apparent anomaly.

Johnson studied the central nervous system of a prawn, a shrimp and a crayfish, and found that the largest fibres in the central nervous system form a 'giant-fibre system' which has a similar arrangement and presumably a similar function in all of them. It is interesting to compare the crustacean giant-fibre system with that of the annelids, as described by Stough (1926) in the earthworm. The earthworm giant fibres are syncitial structures, formed by the fusion of the processes of many nerve cells, some lying in each segmental ganglion of the nerve cord. The crustacean giant fibres, on the other hand, were said to be the processes of single cells (Johnson 1924). Yet the anatomical arrangement of the crustacean fibres has many points of resemblance to that found in the earthworm, and it seems that in both animals the giant-fibre system is responsible for the transmission of the nerve impulses concerned in the production of rapid and simultaneous contraction of muscles all along the body. In the earthworm this causes a rapid shortening of the body, and in the prawn a flip of the abdomen. I have found evidence that the system in the prawn is structurally even closer to that in the earthworm than Johnson's view suggested.

Histological methods

The prawn, *Leander serratus* (Pennant), was the subject of the investigation: it resembles Johnson's prawn *Palaemonetes* very closely in the characters used by systematists. The central nervous system was removed from the animals by dissection under sea water, and before fixation it was tied to glass capillary tubing under natural conditions of tension. For the demonstration of the myelin sheaths of the fibres the material was fixed in a solution containing osmium tetroxide which penetrated the nervous system completely and black-

ened the lipides of the myelin sheaths. The fixative used was either osmium tetroxide 0·2 % in sea water or osmium tetroxide 0·2 % in a saturated solution of picric acid in sea water. Both these fixatives have the disadvantage that they often give a very unlifelike preservation of the axons of the fibres, for the axoplasm was commonly distorted and shrunk, leaving an unnatural space between the axon and the myelin (figure 14, plate 22). The myelin sheaths were, however, very well shown, and it is clear from the illustrations to Johnson's paper that a similar distortion was often given by the osmium fixative he thought best, a mixture of picric and acetic acids with osmium tetroxide and platinic chloride. Flemming's fluid also was not a good fixative for the axoplasm.

The fibre axoplasm is much better preserved by another fixative, a saturated solution of picric acid in sea water. This simple fixative was recommended by Young (1939) for the giant axons of cephalopods, and in the prawn material also the axoplasm was homogeneously fixed, and there was no sign of shrinkage or distortion if the after-treatment of the material was optimal. Figure 3, plate 22, shows in longitudinal section a giant fibre that has been fixed in this solution. The myelin is not preserved by the picric acid, and most of it is removed by fat solvents during dehydration and embedding.

For embedding the celloidin method was sometimes used, with very satisfactory results (figures 3, 15, plate 22). However, the methyl-benzoate, celloidin, paraffin technique of Péterfi was usually employed, for it is almost as successful in avoiding shrinkage of the axoplasm, and has the advantage that thin serial sections can be cut. Ordinary paraffin embedding in which the tissue is transferred to wax from the clearing agent often caused serious distortion, and was therefore avoided.

Serial transverse and longitudinal sections of the nervous system were cut. Safranin in aniline water was found to be the best nuclear stain for the osmium-fixed material, and as it also gave a coloration to the cytoplasm no counterstain was necessary. After picric acid fixation the most useful preparations were given by staining with Heidenhain's azan modification of Mallory's trichrome method; Ehrlich's haematoxylin and eosin were also used. The finer crustacean collagen fibres seem not to have as sharp an affinity for anilin blue as similar fibres in mammalian material, so that the demonstration of collagen in the trichrome preparations was not always uniform.

None of these stains demonstrates fine non-myelinated axons or the fine processes of the larger axons, so that alone they give a very incomplete picture of the nervous system. I found, however, that Bodian's (1936) method for the demonstration of nerve axons with activated Protargol gave very satisfactory results when applied to the mounted sections of the picric-fixed material, all the remaining picric acid being removed from the sections with alcohol before impregnation. The advantage was thus given of a specific impregnation of axons and their finest processes in material of which the fixation was known to be a satisfactory preservative of the axons: this consideration is of great importance when the structure of synaptic connexions is to be studied.

The anatomy of the giant fibre system

The general anatomy of the giant-fibre system in *Leander* does not show any significant variations from the condition *Palaemonetes* so far as Johnson described it, and his account

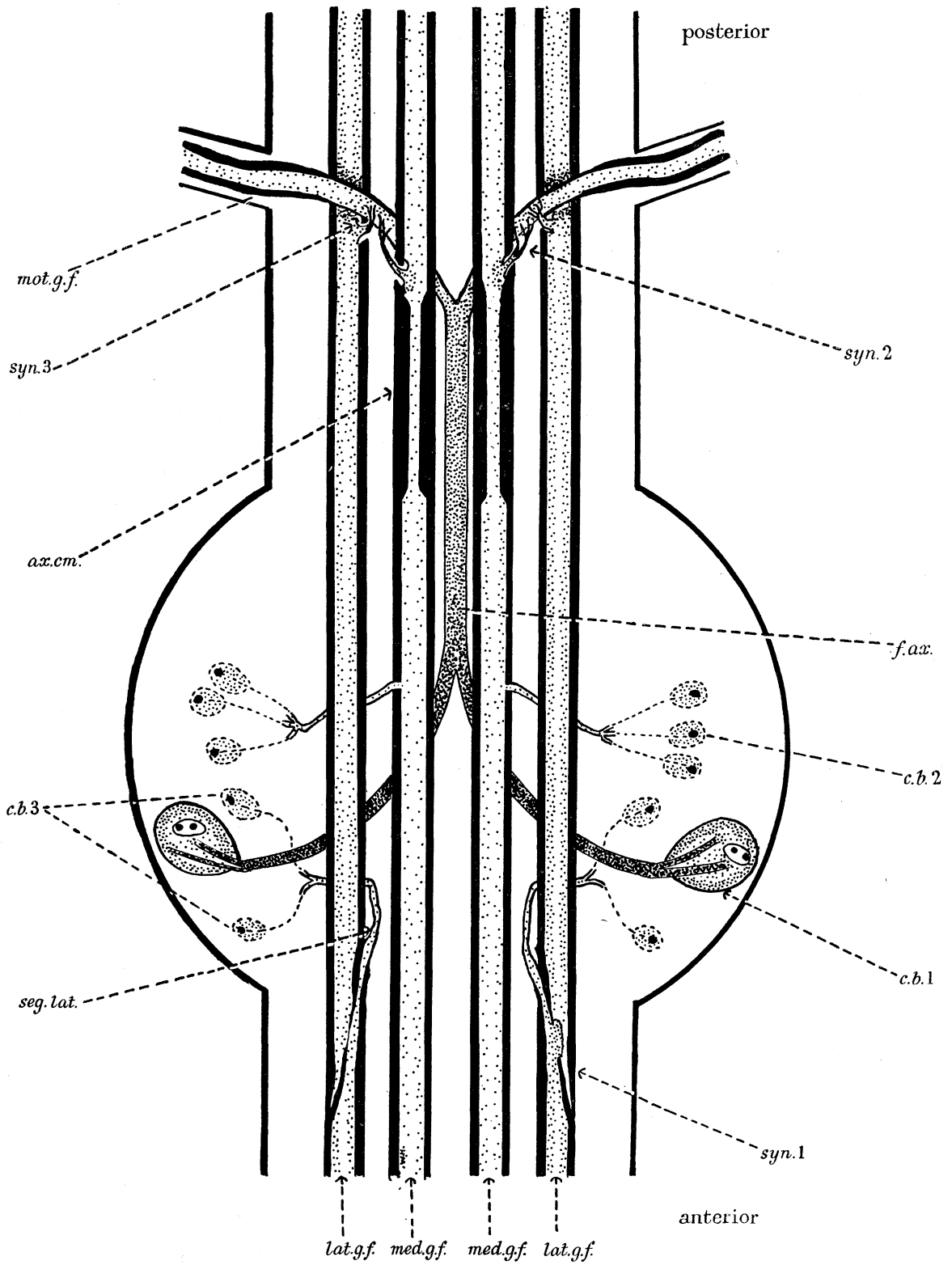


FIGURE 44. Diagrammatic dorsal view of the arrangement of the giant fibres in an abdominal ganglion of the central nervous system of *Leander serratus*. The myelin sheaths of the fibres are represented as dense black lines; the axoplasm is stippled. The number and location of the cell bodies of the median and lateral fibres is hypothetical, and their connexions with the fibres, which have not been demonstrated, are represented by broken lines. *ax.cm.* = segmental constriction of the axon of the median giant fibre; *c.b.*1 = cell body of the motor giant fibre; *c.b.*2 = cell bodies of the median giant fibre; *c.b.*3 = cell bodies of the lateral giant fibre; *f.ax.* = single axon produced by the fusion of the motor giant fibres; *lat.g.f.* = lateral giant fibre; *med.g.f.* = median giant fibre; *mot.g.f.* = motor giant fibre; *seg.lat.* = the lateral giant fibre arising in the segment; *syn.*1 = synapse between two successive lateral giant fibres; *syn.*2 = synapse between a median and a motor giant fibre; *syn.*3 = synapse between a lateral and a motor giant fibre.

may be consulted for further detail. The following is a brief summary of the arrangement of the giant fibres in an abdominal ganglion and the adjacent cord in *Leander*; it is illustrated by figure 44 which also embodies some new observations. The description can be taken as typical for each segment of the ventral nerve cord, though the Crustacea show segmental variations from type in the central nervous system as well as in other morphological characters. This variation is most marked in the anterior thoracic ganglia.

The median pair of giant fibres passes dorsally in the central nervous system from the supraoesophageal ganglia to the last segment of the abdomen, one fibre lying on each side of the middle line (figure 1, plate 22; figure 44). One fibre passes down each circumoesophageal connective. Johnson named this pair the median giant fibres, and considered that each is the axonic process of a single large nerve cell in the supraoesophageal ganglia, though he did not trace them. The conduction velocity of these fibres has been found to be of the order of 20 m./sec. (Holmes *et al.* 1941), and there is evidence that they convey nerve impulses which cause the almost simultaneous contraction of the flexor muscles of the abdomen by the excitation of the motor giant fibres with which they come in contact in each segment (Wiersma 1938).

The motor giant fibres are segmental: a pair of them arises in each segment and is confined to that segment passing out laterally in one of the peripheral nerves. Johnson stated that in *Palaemonetes* the members of the pair fuse together in the mid-line in the ganglion, forming a single median fused axon. This then divides again posteriorly (figure 44) and the two motor axons pass out to the peripheral musculature after entering into synaptic relations with the median and lateral giant fibres.

The lateral longitudinal giant fibres (figure 1, plate 22; figure 44) are a pair lying dorsolaterally and, like the motor axons, are segmental in origin; but, unlike the motor axons, they are confined to the central nervous system, and the lateral giant fibre of one ganglion passes through the interganglionic cord and enters into relations with the lateral giant fibre of the next segment. Thus a chain of fibres is formed along the nerve cord, and it is at the point of contact between the members of the chain that Johnson described the unusual synaptic relationship.

The structure of the nerve fibres

The prawn myelinated fibres are surrounded by a connective tissue sheath whose density is comparable with that of the endoneural connective sheath of vertebrate nerve (figure 45). The fibres in this sheath impregnate sharply by a silver method (Robb-Smith 1937) specific for reticulin fibres in vertebrate connective tissue. The nerve fibres differ from those of vertebrates in having a nucleated inner sheath between the axon and the myelin layer and in having no Schwann nuclei between the myelin layer and the connective tissue (figure 45).

The inner sheath and the inner nuclei

After picric acid fixation and the consequent disappearance of lipides from the myelin layer a thin nucleated fibrous sheath can usually be seen around the surface of the axon of the giant fibres (figures 4, 6 and 12, plate 22). A similar sheath has been seen in other

crustacean and cephalopod nerves between the 'metatropic' myelin-containing layer and the axon (Young 1936). In these picric acid preparations the myelin-containing layer is represented by a sparse network, presumably of protein composition; and the fibres of this network appear to be continuous with those of the inner sheath and the connective tissue endoneurium (figures 3 and 12, plate 22).

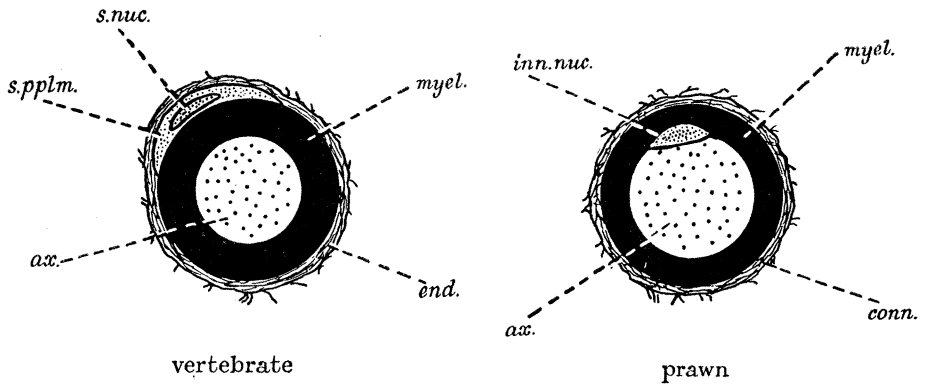


FIGURE 45. Diagrammatic transverse sections of myelinated fibres of a vertebrate and a prawn. In the vertebrate fibre the Schwann sheath between the endoneurium and the myelin is not shown. *ax.* = axon; *conn.* = connective tissue sheath corresponding to vertebrate endoneurium; *end.* = endoneurium and Schwann sheath; *inn.nuc.* = nucleus between axon and myelin; *myel.* = myelin sheath; *s.nuc.* = Schwann nucleus; *s.pplm.* = Schwann cytoplasm.

If the inner sheath and the neurokeratin network are held to be formed within or by the activity of the 'inner sheath cells', then these cells are comparable with the Schwann cells of vertebrates. The inner nuclei would be the nuclei of the myelin sheath, the inner sheath being merely that part of the myelin sheath in which there was a high ratio of protein to lipide. In the rest of the sheath there is much more lipide than protein, the latter forming the neurokeratin network. Ramifications of the inner cell cytoplasm may extend within the myelin layer just as the Schwann cell cytoplasm has been said to do in vertebrates (see Doinikow 1911).

Thus the 'inner sheath cells' may be considered to play the same rôle as Schwann cells in myelin sheath formation and in the structure of the adult nerve. On the other hand there is no evidence to exclude the description of the inner sheath as an independent connective tissue layer, though it has no marked affinity for anilin blue, and does not impregnate by the reticulin method. It is relevant to bear in mind similar difficulties which have arisen in the study of the relations of the vertebrate Schwann sheath or neurilemma with the Schwann cells and connective tissue endoneurium (see Holmes and Young 1942).

However the presence of a protein layer between axon and myelin remains a difference between vertebrate and invertebrate myelinated fibres; and this layer may well have a significant rôle in the process of impulse conduction.

The myelin sheath

The data on the distribution of lipides in the sheaths of invertebrate nerve fibres are reviewed by Schmitt & Bear (1939): the lipide is usually demonstrable only by polarized

light analysis, and not by osmium treatment, but the lipide layer is always, as in the prawn, separated from the axon by a nucleated inner sheath.

I have been unsuccessful in attempts to stain the myelin sheaths of the prawn fibres by Weigert's method after mordanting the whole central nervous system in potassium dichromate, which suggests that vertebrate and invertebrate myelin may be qualitatively different.

Almost all of the prawn fibres of a diameter greater than 15μ have their myelin sheath interrupted at intervals by nodes of Ranvier, in which the axon has no myelin covering (figure 13, plate 22). The close similarity between vertebrate and invertebrate nodes is of great interest in view of the importance that is attributed to them in the conduction of nerve impulses in myelinated fibres in which the sheath has more than a certain thickness or lipide density (see Offner, Weinberg & Young 1940). It is thus remarkable that I have not been able to find any structures resembling nodes in the sheaths of the giant fibres within the central nervous system. The motor giant fibres, however, although they are non-myelinated within the central nervous system, have nodes in their sheaths in their peripheral distribution. (In their course from the central nervous system to the abdominal muscles the motor giant fibres often have the total diameter of 80μ, so that they are the largest of the giant fibres of the prawn.) The median giant fibres can be followed for some centimetres along the nerve cord, but at no point is their myelin sheath completely interrupted. In each abdominal ganglion, however, they show a structural modification of their axons and sheaths; the diameter of the fibre axon decreases over a distance of 200–400μ to about a third of its normal value. This decrease of axon diameter is not accompanied by a decrease in the total diameter of the fibre, for over the same distance the myelin-containing layer increases in thickness. In transverse sections of picric-fixed material it can be seen that the axon at this point is surrounded by an unusually dense protein component, so that the increase in the thickness of the sheath is due to an increase in the protein elements in the sheath in the region immediately around the axon (figure 12, plate 22). But throughout the region of the axonic constriction the sheath blackens with osmic acid, and is in no sense interrupted. It is interesting to speculate whether this segmental structural change is part of a mechanism for the transmission of nerve impulses along myelinated fibres which have no Ranvier nodes.

In the fibres which are provided with Ranvier nodes the internodal distances vary considerably even along a single fibre (Holmes *et al.* 1941).

Gasser & Grundfest (1939), Schmitt & Bear (1937) and others have studied the relationship between axon diameter and sheath thickness in vertebrate fibres. Definite numerical values for the ratio axon diameter/total diameter have also been established for myelinated fibres of different sizes in the earthworm (Taylor 1940), the prawn (Holmes *et al.* 1941) and the shrimp (Taylor 1941).

In the prawn, however, the motor giant fibres provide a striking exception to the sheath/axon ratio established, for over most of their course within the ganglia they have no myelin sheath, although a thick sheath is present around all other fibres of a similar diameter. From their origin from ventral cell bodies (see p. 300), through the region of fusion and redivision, and at the point of synapsis with the median and lateral fibres they are never provided with sufficient lipide in their sheaths to show any blackening with

osmium tetroxide (figures 16–19, plate 23). But after contact with the other giant fibres myelin appears in their sheaths as they pass to the periphery of the central nervous system, and by the time they leave it they are as heavily myelinated as all other fibres of a similar diameter.

There is reason to believe that the motor giant fibres are excited by the median and lateral fibres at their point of synapsis, transmitting rapid nerve impulses to the abdominal muscles. The motor fibres are thus myelinated only over that part of their course over which they transmit these impulses, and the sheath thickness/axon diameter ratio is not invariable.

Neuronal fusion and synaptic structure

Histological demonstration of a barrier between the cytoplasms of two neurons at their point of contact is not always easy, and much controversy has centred round the problem of whether or not neurons commonly have a syncitial relationship with each other. Supporters of the 'neuron theory' have accumulated a mass of evidence that there is always a separation between the neuroplasms at the synapse, and that neuronal fusion only exceptionally takes place (Cajal 1934). Physiological evidence supports that view, though in a small number of cases the occurrence of neuronal fusion has been verified both histologically and physiologically (Young 1939).

Neuronal fusion—the motor giant fibres

Johnson (1924) stated that the axons of the motor giant fibres of the prawn arise ventrally in the ganglia, pass dorsally and to the mid-line, and then fuse together (figure 44). A single axon is thus formed from the original pair, and this passes along the cord for some distance, and then divides again before leaving the central nervous system for the peripheral musculature (figure 5, plate 22). He did not succeed, however, in tracing the axons back to their cell bodies, and therefore did not dispose of the possibility that the fusion was between two processes of a single neuron.

By the use of material stained by Bodian's method I have been able to trace the motor giant fibres of *Leander* back to their cell bodies and to verify that fusion of the axons of two distinct neurons takes place.

The origin of one motor axon from its cell body is shown in figures 7–11, plate 22. It is formed by the fusion of two distinct intracellular axons (see Lacroix 1935). Two axonal processes arise from within the cytoplasm of each nerve cell: only after leaving the cell body do they fuse to form a motor giant fibre. One axon is formed in this way ventrally on each side of the ganglion; the two then follow the course already described until they come together dorsally in the mid-line. They first lie together separated by their sheaths (figure 26 *a*, plate 23): then the two axons lie within a common sheath (figure 26 *b*, plate 23), and as the series of transverse sections is followed complete fusion can be seen to take place between the axons, and a single round axoplasm with a sheath exactly like that of a single fibre is formed (figure 26 *c* and *d*, plate 23). No structure or membrane is visible within this axoplasm, and no histological separation between the two fused axons is demonstrable, for the whole stains with complete uniformity. After passing along the cord this axon divides into two, one axon passing out as described on either side of the body.

Synaptic structure—the lateral giant fibre synapses

Johnson (1924) stated that at the lateral giant fibre synapses in the shrimp *Crangon* the cytoplasm of the two fibres concerned was continuous, but that their neurofibrillae remained separate. In the prawn *Palaemonetes* 'a membrane is found to separate the fibers for part of the region of contact, but this cannot be seen in the anterior portion of this region and... the neurofibrillae of one fiber spread out somewhat within the cytoplasm of the other at the point where the membrane is not seen'. 'The study of these conditions... appears to me to make clear that relations between neurons may be by contact with a membrane intervening, by contact without an intervening membrane, and with the spreading of neurofibrillae of one fiber a short distance into the axis cylinder of the other.'

For the study of the fine structure of synapses the use of cytological fixatives is essential, as Bodian (1937) has shown by the description of the artefacts produced by poor fixation of the synaptic endings on Mauthner's cell in the goldfish. Johnson's osmic acid fixative produced considerable shrinkage of the axoplasm of the prawn fibres, and it appears that he used the word 'neurofibrils' in an unusual sense, for in his figures there are no structures resembling neurofibrils as they are usually demonstrated by silver impregnation. In my material fixed in picric acid Bodian's method does not reveal any neurofibrillar structure in the axons.

Picric acid fixes the axoplasm of the giant fibres without shrinkage or distortion, so it may reasonably be described as a cytological fixative for their synapses. And as the prawn ganglia are never more than 1 or 2 mm. in diameter the whole piece should be fixed rapidly enough to avoid the post-mortem artefacts which Bodian described in large pieces of nervous tissue fixed by immersion.

If the lateral giant fibres are followed along the nerve cord in serial transverse sections it can be seen that each fibre arises in a ganglion and lies in synapsis side by side with the anterior end of the fibre arising in the ganglion next posterior (figure 44). It then passes anteriorly through the connective to the next ganglion where it enters into synapsis with the lateral fibre arising there. It then gradually decreases in diameter and comes to an end. The distance over which the synapsing fibres lie together is very variable; a mean value for it is 150μ.

The synapses show much variation in different parts of the nerve cord, but the essential features of sheath and axon structure are the same in all of them, and it is clear after picric acid fixation that there is always histological evidence of a complete separation of their axoplasms throughout the region of contact.

In some cases a definite membrane, similar in staining reactions to the protein basis of the myelin sheath and apparently continuous with it, is visible between the axons at all points (figures 32–36, plate 24). In other cases this membrane may disappear over a distance of some 10μ, but even where it is not present there is a sharp staining difference between the axoplasms clearly marking off one from the other and indicating a separation between them. This separation can also be seen in the osmium-fixed material, and in this the distribution of myelin at the synapse can be followed. The membrane which separates the axoplasms contains sufficient fat to blacken with osmium tetroxide over almost the whole of the region of association of the fibres (figures 37–43, plate 24). In the synapse

figured in the plate such a black-staining membrane is absent only in one section 8μ thick, and even in this there can be no doubt that the axoplasms are separate, even though that of one fibre passes within the sheath of the other (figure 39, plate 24).

At the lateral giant-fibre synapses, therefore, the axoplasms remain distinct. Throughout the greater part of their association they are separated by a lipide-containing layer which appears to be continuous with the myelin sheath, and even in cases where no lipide or protein sheath is visible differences in staining capacity show that the axoplasms do not mingle. Similar staining differences between axons at a synapse have been observed in cephalopods by Young (1939). It can be concluded that there is nothing in the structure of the lateral giant-fibre synapses in *Leander* to justify the creation of a new category of neuronal relationships to include them.

The synapses between the motor fibres and the median and lateral fibres

Johnson pointed out that in the prawn the motor giant fibres, after their redivision from the fused axon and before they leave the cord, enter into relations of contact with the median and lateral fibres (figure 44). I have examined these synapses in longitudinal and transverse sections by the various histological methods described. The most complete picture of them is given by the silver impregnation method, for the others do not show the fine axonic processes that are concerned.

In passing to the periphery of the cord each motor fibre lies below the median fibre and above the lateral fibre: as has already been stated the motor fibres have no myelin sheath in this region. The myelin sheath of the median and lateral fibres is modified at the point where the motor fibres are closest to them by the appearance in it of a small gap not more than 15μ in diameter (figures 16–19, plate 23), and through this 'hole' in the sheath the

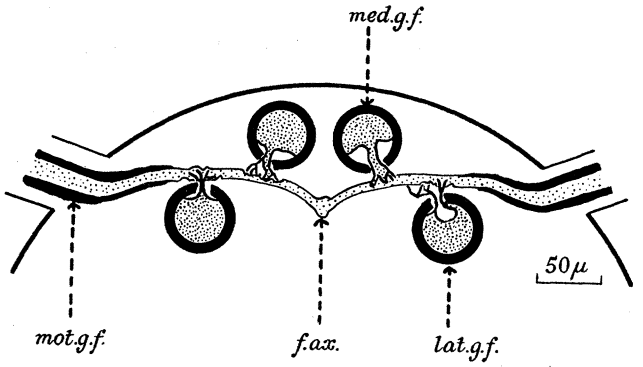


FIGURE 46. Transverse section through the dorsal part of an abdominal ganglion showing the synapses of the median and lateral fibres with the motor fibres. *med.g.f.* = median giant fibre; *mot.g.f.* = motor giant fibre; *lat.g.f.* = lateral giant fibre; *f.ax.* = median fused axon.

axoplasm of the fibres puts out processes, some of them thick and blunt, others fine and branching. The axoplasm also seems not completely to fill the sheaths at this point, though the appearance may be an artefact (figures 21, 22, plate 23). These processes terminate on the surface of the axon of the motor fibre (figure 46; figures 21–23, plate 23). From the histological appearances it is not always possible to state that the relation of the finest of these processes with the motor axon is one of synapsis; but the larger processes can be seen

to remain separate from the motor axon; and even when a definite membrane of separation cannot be seen the axoplasm of the motor fibre is usually distorted by irregularities of its surface, and these almost certainly represent the fixation picture of fine membranes or surfaces of separation (figure 21, plate 23). Thus the evidence suggests that the relationship is one of true synapsis.

The median and lateral fibres: unicellular neurons or syncitia?

Johnson believed that the cell bodies of the median giant fibres in the prawn and other Crustacea are located in the supraoesophageal ganglia. This view he held partly by analogy with certain giant axons in lobster embryos, arising from giant cells in the brain and passing along the nerve cord (Allen 1894), and partly on degeneration experiments on the giant fibres of *Cambarus*. He transected the nerve cord of the crayfish at various levels and examined it histologically some weeks later (Johnson 1926). His experiments cannot be considered as conclusive proof of the cerebral situation of the median fibre cell bodies, for after several weeks he found only 'a slight amount of degeneration', somewhat more marked posteriorly than anteriorly to the cut, and in a small proportion of his experimental animals.

In mammals degenerative changes begin very soon in a nerve fibre that is cut off from its cell body: the axon begins to break up within 24 hr. of the injury, and degenerative changes in the myelin sheath follow soon afterwards. There is no reason to believe that isolated invertebrate axons can persist indefinitely. Mapelli (1931) described degeneration in the nerve cord of a crayfish. After section of the cord degeneration of those axons that are separated from their cell bodies takes place, and later regenerative outgrowths from the central stumps are seen as in vertebrates. This author gives no accurate data on the rate of degeneration, but Sereni & Young (1932) found that in cephalopods the rate of degeneration at summer temperature at Naples is much the same as that found in mammals. It thus seems reasonable to expect that the crustacean fibres would show much more degeneration than Johnson found if they had been separated from their cell bodies for several weeks. However, the degeneration of myelinated fibres in cold-blooded vertebrates, as described by Mönckeberg & Bethe (1899) in the frog, is much slower than in warm-blooded animals, so that the possibility that the large prawn fibres degenerate very slowly cannot be excluded. But Johnson found that if the nerve cord of *Cambarus* was cut posteriorly to the second abdominal ganglion, and the animal kept alive for 14–45 days, electrical stimulation of the anterior end of the isolated posterior part of the cord resulted in the animal giving an abdominal flip which was as strong as that given by control animals. It is difficult to accept his conclusion that there had been no loss of function of the median giant fibres several weeks after they had been separated from their cell bodies.

Experimental degeneration of the median giant fibres

The nerve cord in a number of prawns was transected at various levels by means of fine scissors passed through a small aperture made by a sharp pointed knife at an appropriate point in the chitin. Figure 47 shows the different levels at which the cord was cut in different animals. The prawns were killed at various intervals after the operation and their

nervous systems removed for histological examination. In some cases one circumoesophageal connective was cut, so that only one median giant fibre was interrupted. In one case two cuts were made in the cord in the abdomen, so that a length of cord containing two ganglia was isolated.

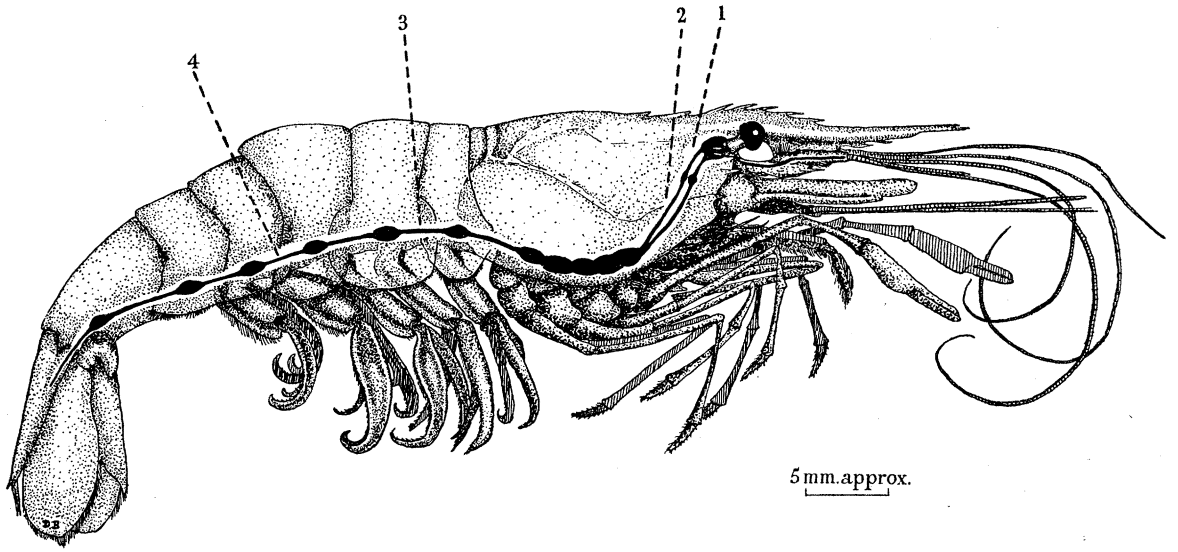


FIGURE 47. Lateral view of *Leander serratus*, the central nervous system being shown by transparency. The numerals indicate the levels of the experimental lesions described in the text.

The nerve cord was examined by teasing and fixation in osmium tetroxide, and in serial sections after picric and osmium fixation.

Three days after the transection of one circumoesophageal connective marked degenerative changes are seen in the myelin sheaths of the smaller fibres, but none in the giant fibres that have been cut off from the brain. In teased preparations it can be seen that the myelin has collapsed, forming chambers in which the remains of the axon are contained, in a way very similar to that seen in early myelin degeneration in vertebrates (figure 48*a*). Eleven days after a similar transection the myelin of the fibres in the connective is still further collapsed and the axon remains are more completely obliterated (figure 48*b*).

This degeneration may be said to be comparable with traumatic degeneration in higher animals; that is, the degeneration which goes on in the region of the lesion and as a direct result of the trauma and inflammation resulting from it. However, true 'Wallerian' degeneration of prawn fibres does take place, for 11 days after section of the connective degenerative changes can be found in the posterior region of the abdomen, remote from the injury.

I have not seen any sign that the 'inner sheath' cells proliferate in the distal part of the divided cord as do the Schwann cells after lesions of vertebrate peripheral nerve. It is difficult, however, to prove this negative view, as proliferation can only be certainly established when an extensive degenerating tract of nerve fibres can be compared with a normal one. And of course there are no such tracts in the prawn cord passing sufficiently far along the cord to exclude the possibility that an increase in the number of cells present is due to invasion of the tissue by phagocytes from the region of the injury. It has been suggested that the 'inner sheath' cells may be homologous with the Schwann cells of verte-

brates (p. 298), so that this point is worth investigation. A clear result might be given by the production of experimental lesions of the motor nerves to the abdominal muscles or thoracic appendages.

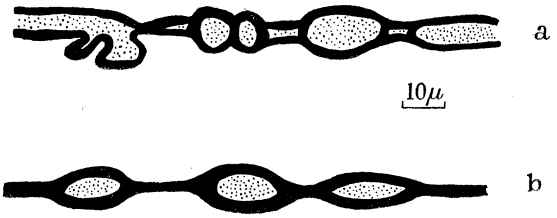


FIGURE 48. Degenerative changes in the myelin sheath of small prawn fibres. (*a*) A fibre from the posterior region of the circumoesophageal connective 3 days after a lesion at the anterior end of the connective. (*b*) A similar fibre 11 days after the lesion. The figures were drawn from material fixed and teased in an 0·5 % solution of osmium tetroxide in sea water.

The observations on degeneration so far described were made on the smaller fibres in the nervous system. When the median giant fibres were examined in serial sections a very different result was seen.

A median giant fibre that has been cut at any point in the nervous system degenerates only over a distance of some 3 mm. from the injury: this is presumably traumatic degeneration. In a prawn in which one circumoesophageal connective was transected at level 2 in figure 47 the median giant fibre was found 12 days later to have disappeared for a short distance into the first thoracic ganglion, leaving no identifiable remains on the injured side (figure 27, plate 24). But in a transverse section taken a little farther back in the first thoracic ganglion the giant fibre can be seen again (figure 28, plate 24), and in the posterior region of the ganglion, where presumably it was uninjured by the direct trauma, it can be seen to be perfectly normal, with a uniform axoplasm, and no sign of sheath abnormality (figure 29, plate 24). And in the rest of the series of sections of this fibre in the cord posterior to the cut its axon is found to be continuous and without any interruption or fragmentation, although it had been separated from the supraoesophageal ganglia for 11 days.

When one circumoesophageal connective was transected at level 1 anterior to the commissural ganglion, the commissure posterior to this ganglion was found 14 days later to be completely degenerate in that the myelin sheath of all the small fibres had been destroyed and no longer blackened normally with osmium tetroxide, but the median giant fibre it contained was of normal appearance and its myelin sheath blackened normally.

In the animal in which a portion of the cord containing two ganglia was isolated for 14 days by cuts at levels 3 and 4, the isolated cord was found to contain median giant fibres whose appearance was not significantly modified from the normal.

I was able to confirm Johnson's observations on electrical stimulation of the nerve cord in that I found that animals whose circumoesophageal connectives had both been cut were still capable 14 days later of giving a normal abdominal flip when stimulated by compressing the body in the thoracic region.

It seems reasonable to conclude from these experiments that all the cuts made in the central nervous system failed to isolate the median giant fibres from their cell bodies. Thus they must be syncitial structures formed by the fusion of the processes of cell bodies of

which some are probably located in each of the ganglia of the central nervous system. Only such a syncitial axon would fail to degenerate when cut at any point, for both the parts of the median giant fibre separated by a cut behave like the central stump of a divided vertebrate nerve.

It must, however, be emphasized that in some of the degeneration experiments this conclusion depends on the assumption that the giant fibres react to trauma and to separation from their cell bodies at the same rate as do the smaller myelinated fibres, and that they are not specially resistant to degeneration. Proof of the assumption cannot be given, and it is possible that their integrity is due to this difference.

The cell bodies of the median and lateral giant fibres

The single large cell bodies of the motor giant fibres and their axons are easily followed out in serial sections, but I have not been successful in locating those of the median and lateral fibres. This is because their connexions with the giant axons are through very fine processes, and the tracing of such through serial sections is extremely difficult.

The examination of serial sections of the supraoesophageal ganglia, stained by Bodian's method, shows that the anterior termination of the median giant fibres is in fine processes which appear to be subdivisions of the giant axon. These cannot be traced to cell bodies, but there is no evidence that the median fibres are connected with giant cells in these ganglia as Johnson suggested. In each ganglion of the central nervous system in the abdomen, and probably in the thorax also, axons that are never more than 4μ in diameter, and usually much smaller, pass through the sheaths of the median fibres and appear to fuse with their axons (figure 2, plate 22; figures 24, 25, plate 23). In many cases it is clear that these processes are in true fusion with the median axon, and they can be traced back laterally and ventrally in the ganglion towards the nerve cell bodies in it.

The lateral giant fibres which arise in each ganglion do not arise from a single axon of the dimensions of the main lateral fibre, and it seems that they also are formed by the fusion of several very small axons.

A histological proof that the median and lateral giant fibres are syncitial structures thus cannot be given, but the fact that the axons can never be traced back to single cell bodies in spite of their large size, and the fact that they enter into relations of apparent fusion with axons in each ganglion, seems ample proof that they are fused neurons and, unlike the motor fibres, connected with more than one cell body (figure 44).

The septa in the median giant fibres

In the thoracic ganglia of the ventral nerve cord the axoplasm of each median giant fibre is frequently interrupted by septa which pass through the axoplasm. These septa never, however, divide the axoplasm completely, for although they may appear to do so in one section when they pass from one side of the fibre to the other, it is found on following the series that they are at some point incomplete (figures 30, 31, plate 24; figure 4, plate 22), so that the axoplasm of the median fibre in fact passes without interruption from one end of the cord to the other. The septa are of variable appearance though they always seem to be continuous with the inner layer of the inner sheath around the axon; that is, perhaps,

with the cytoplasm of the inner sheath cell. Sometimes they are thin and membraneous (figures 30, 31, plate 24); at other times they are thicker and show a fibrillary structure (figure 4, plate 22), though the fibrils are never so coarse as those of the neurokeratin or the endoneurium.

That there is true continuity of the axoplasm past these septa is shown not only by the absence of any complete separating membrane, but also by the absence of any staining difference on the axon or any other of the appearances of synaptic separation such as are seen at the contacts in the lateral giant fibre chain. These septa are found only rarely in the abdominal ganglia.

Discussion

An interesting comparison can be made between the giant-fibre system of the prawn and that of the earthworm. In the latter animal three giant axons, provided with myelin sheaths, pass along the ventral nerve cord. They are not, however, continuous; for in each segment the axoplasm is traversed by a septum, so that the giant fibres are in fact made up of a chain of segmental axons like the lateral fibre system in the crustacean. The axoplasm of each segmental fibre is completely separated by the septum from that of the next fibre in the chain. Each fibre of the chain has been shown to be a syncitium, for they are made up by the fusion of the processes of several nerve cells in the corresponding ganglion. The syncitial nature of the earthworm axons has been proved by tracing the axons which compose them back to their cell bodies, and by degeneration experiments in which it was shown that the giant fibres do not degenerate on either side of any cut made in them (see Stough 1926).

The results of the degeneration experiments here described are not a conclusive proof that the prawn median and lateral axons also are syncitial in structure, but they are reinforced by the observation of apparent fusions between the large axons and small collaterals in each ganglion. And the fact that the median and lateral fibres are the only large axons not provided with typical Ranvier nodes is further evidence of their structural dissimilarity from the axons having single cell bodies. It has been noted in cephalopods that some giant fibres are formed by the fusion of the axons of many small cell bodies, while others equally large are the processes of single giant cells (Young 1939), a distinction which would be similar to that between the prawn median and lateral fibres on the one hand and the motor fibres on the other. It may be concluded that the median and lateral prawn fibres are probably like the earthworm giant fibres in being syncitial structures: the median fibres differ from those of the earthworm, however, in passing with a continuous axoplasm from the supraoesophageal ganglion to the last abdominal segment of the nerve cord. Stough noticed that in one or two cases the septa dividing the axons in the earthworm giant-fibre chain were incomplete, so that the axoplasm of one segment was continuous with that of the next. There is a striking similarity between these incomplete septa found rarely in the earthworm and the incomplete septa which occur regularly in the prawn, where the median fibres are never completely divided up. It can thus be suggested that the incomplete septa in the thoracic cord of the prawn are relics of segmental synapses between successive elements in a chain of fibres. These have broken down so that the whole chain has become a syncitial unit. It may be that in the develop-

ment of the individual prawn the median fibre arises first as a chain of separate axons, and that during the animal's lifetime the synapses between them break down and are seen at different stages of their degeneration in different animals or at different levels in the nervous system. Or it may be that these incomplete septa are retained in the modern prawn because they play some part in nerve impulse conduction. This view suggests itself because the septa are found in the thoracic cord and are rarely present in the abdomen, while the segmental constrictions occur regularly in the abdomen and seldom in the thorax. The possibility that the latter play the part of nodes in the conduction process has already been mentioned.

Synaptic structure

In *Leander serratus* the axoplasms of the lateral giant fibres are distinct at their synapses, and I find no evidence of neurofibrillar or other fusion. But even if a condition such as Johnson described in *Palaemonetes* is established, it cannot be said to be a new type of relationship between neurons. His 'synapse with partial fusion' is very like the incomplete septa in the median fibres of *Leander*, and such an arrangement cannot be described as a synapse. There is every probability that nerve impulses will be transmitted without interruption past the incomplete septum over the surface of the fused axons: it is most unlikely that it would function physiologically as a synapse, for in all cases where a synapse has been demonstrated physiologically complete axonic discontinuity has been convincingly proved.

Cajal (1934) declared that he had never seen synapses between axons, but these must be common in invertebrates, where so many of the neurons are unipolar; that is, without dendrites. Two very different types of axo-axonic synapses are described here in the prawn. The lateral giant-fibre synapses, which are between two myelinated axons, are symmetrical, for there is no difference in morphology or surface area between the axons concerned. The two fibres lie side by side for a considerable distance, but only over a very small part of it are their axoplasms not separated by a myelin sheath. If the nerve impulse is only transmitted from one axon to the other over the non-myelinated surface between them it is not clear why the long side to side association should take place. It may be that most of the axonic surfaces in the region of association are concerned in the transmission of the impulse, even though they are separated by an 'insulating' myelin layer.

The synapses of the median and lateral fibres with the motor fibres are of a different kind: they are between myelinated and non-myelinated axons and resemble more the axo-dendritic synapses of vertebrates. The median and lateral axons send out many fine processes through relatively minute gaps in their myelin sheaths, and these processes terminate on the surface of the motor axon. No 'boutons terminaux' or other specialized endings to these processes have been observed. Such a synapse can probably only conduct impulses in one direction; that is, from the median and lateral fibres to the motor fibres: the physiological differences between symmetrical and asymmetrical synapses were discussed by Young (1939). A recent paper by Bodian (1942) should be consulted for a valuable review of the cytology of synapses in relation to their function.

Vertebrate and invertebrate myelinated fibres

The observation that most of the larger prawn fibres have nodes in their myelin sheath is clear evidence that nodes play an important part in impulse conduction in myelinated fibres. In their structure the vertebrate and invertebrate nodes are significantly alike, for both involve the exposure of the axon over a short distance in which no osmium-staining fat covers it. If the segmental constrictions in the median fibres play a part in impulse conduction in the absence of nodes, then the temporary decrease in the diameter and surface area of the axon and the decrease in the ratio of lipide to protein element in the sheath immediately surrounding it must in some way serve to transmit the impulse from one adjacent normal segment of the giant fibre to the next, playing the same part as do the nodes in the other fibres.

It may be concluded that the giant fibres of the prawn have no characteristics that are not compatible with accepted views on the structure and relations of neurons, but they offer several special features the study of which may advance our knowledge of the mechanism of impulse transmission in myelinated fibres.

The animals were collected and the experimental work done at the Laboratory of the Marine Biological Association at Plymouth, and I am grateful to the Director of the Laboratory and to his staff for their hospitality. The histological work was done at the Department of Zoology in Oxford during my tenure of a Christopher Welch Scholarship of the University and a Senior Demyship of Magdalen College. I am indebted to Professor Goodrich for permission to use the Oxford table at Plymouth and to work in his Department. Mr J. Z. Young has given me most valuable advice at every stage of the work.

References

Allen, E. J. 1894 *Quart. J. Micr. Sci.* **36**, 461.
Bodian, D. 1936 *Anat. Rec.* **65**, 89.
Bodian, D. 1937 *J. Comp. Neurol.* **68**, 117.
Bodian, D. 1942 *Physiol. Rev.* **22**, 146.
Cajal, S. R. 1934 *Trab. Lab. Invest. biol. Univ. Madr.* **29**, 1.
Doinikow, B. 1911 *Histol. histopath. Arb.* **4**, 445.
Friedländer, B. 1889 *Mitt. zool. Sta. Neapel*, **9**, 205.
Gasser, H. S. & Grundfest, H. 1939 *Amer. J. Physiol.* **127**, 393.
Holmes, W., Pumphrey, R. J. & Young, J. Z. 1941 *J. Exp. Biol.* **18**, 50.
Holmes, W. & Young, J. Z. 1942 *J. Anat., Lond.* **77**, 1.
Johnson, G. E. 1924 *J. Comp. Neurol.* **36**, 323.
Johnson, G. E. 1926 *J. Comp. Neurol.* **42**, 19.
Lacroix, P. 1935 *Cellule*, **44**, 251.
Mapelli, P. 1931 *Arch. zool. (ital.), Napoli*, **15**, 127.
Mönckeberg, G. & Bethe, A. 1899 *Arch. mikr. Anat.* **54**, 135.
Nageotte, J. 1916 *C.R. Soc. Biol., Paris*, **79**, 259.
Offner, F., Weinberg, A. & Young, G. 1940 *Bull. Math. Biophys.* **2**, 89.
Retzius, G. 1890 *Biol. Untersuch.* n. F. 1.
Robb-Smith, A. H. T. 1937 *J. Path. Bact.* **45**, 312.

Schmitt, F. O. & Bear, R. S. 1937 *J. Cell. Comp. Physiol.* **9**, 261.
Schmitt, F. O. & Bear, R. S. 1939 *Biol. Rev.* **14**, 27.
Sereni, E. & Young, J. Z. 1932 *Pubbl. Staz. zool. Napoli*, **12**, 173.
Stough, H. B. 1926 *J. Comp. Neurol.* **40**, 409.
Taylor, G. W. 1940 *J. Cell. Comp. Physiol.* **15**, 363.
Taylor, G. W. 1941 *J. Cell. Comp. Physiol.* **18**, 233.
Wiersma, C. A. G. 1938 *Proc. Soc. Exp. Biol., N.Y.*, **38**, 661.
Young, J. Z. 1936 *Proc. Roy. Soc.* B, **121**, 319.
Young, J. Z. 1939 *Phil. Trans.* B, **229**, 465.

Description of Plates 22–24

(All the illustrations are of the nervous system of *Leander serratus*)

Plate 22

Figure 1. Transverse section of the central nervous system between two ganglia in the abdomen. Osmium tetroxide fixation.

Figure 2. Transverse section of median giant fibre in abdominal cord, showing an association between its axon and that of a smaller fibre. Fixation formaldehyde-sea water: material mordanted with potassium dichromate for a myelin sheath stain, but this has not been successful.

Figure 3. Longitudinal section of motor giant fibre immediately after leaving the central nervous system in the thoracic region. This fibre at this point is larger than any within the central nervous system. Picric acid fixation, celloidin embedding, stain haematoxylin and eosin.

Figure 4. Transverse section of median giant fibre in a thoracic ganglion, showing an incomplete septum. The septum has a fibrillary appearance. Picric acid fixation, stain haematoxylin and eosin.

Figure 5. Horizontal longitudinal section of the cord in the abdomen showing the division of the median fused axon into the two motor giant fibres, and the path of these out of the cord. Picric acid fixation, Bodian stain.

Figure 6. Transverse section of median fibre showing a nucleus between myelin and axon and the relations of the 'inner sheath'. Picric acid fixation, Mallory-azan stain.

Figures 7–11. Successive transverse sections showing the origin of a motor fibre from a double axon in its large cell body. Figure 11 shows the two axons of origin about to fuse to form the motor fibre. Picric acid fixation, Bodian stain.

Figure 12. Transverse section of two median fibres in an abdominal ganglion. One of the fibres is of normal appearance; the other shows the segmental constriction of the axon with thickening of the inner sheath. Picric acid fixation; Mallory-azan stain.

Figure 13. Whole mount of single teased fibre showing a Ranvier node, from the chela of the largest thoracic appendage. Fixation osmium tetroxide; preparation mounted in glycerin.

Figure 14. Longitudinal section of large fibre from the central nervous system, showing axonic shrinkage after osmium fixation. Fixation picro-osmic mixture.

Figure 15. Longitudinal section of large fibre from the central nervous system showing the relation of the inner nucleus to the axon. Picric acid fixation, celloidin embedding, haematoxylin and eosin stain.

Plate 23

Figures 16–19. Successive sections, 8μ in thickness, showing the synaptic relations between the median and lateral fibres and the motor fibres in an osmium tetroxide preparation. All are to the same scale.

Figures 20–23. Successive sections, comparable with figures 16–19 showing the synaptic relations between the median and lateral fibres and the motor fibres in material fixed in picric acid and stained by Bodian's method. All are to the same scale.

Figure 24. Longitudinal section of median fibre in a ganglion showing its relations with many smaller axons. Picric acid fixation, celloidin embedding, haematoxylin and eosin stain.

Figure 25. Transverse section of abdominal ganglion showing median fibre associated with a small axon, and the first appearance of the lateral fibre of the segment. Picric acid fixation, Bodian stain.

Figure 26 *a–d*. Transverse sections showing the successive stages in the fusion of the two motor axons to form the median fused axon. Picric acid fixation, haematoxylin and eosin.

Plate 24

Figures 27–29. Successive transverse sections of the first thoracic ganglion of the nerve cord in an animal in which one circumoesophageal connective had been transected 12 days previously. The most anterior section, figure 27, shows no trace of the median fibre on the operated side, though that on the other side is normal. Figure 28 is posterior to figure 27 and shows the reappearance of the median fibre on the operated side. Figure 29 is the most posterior section, and shows that in the posterior region of the first thoracic ganglion the median fibre on the operated side is present and normal in appearance. All to the same scale. Picric acid fixation, haematoxylin and eosin.

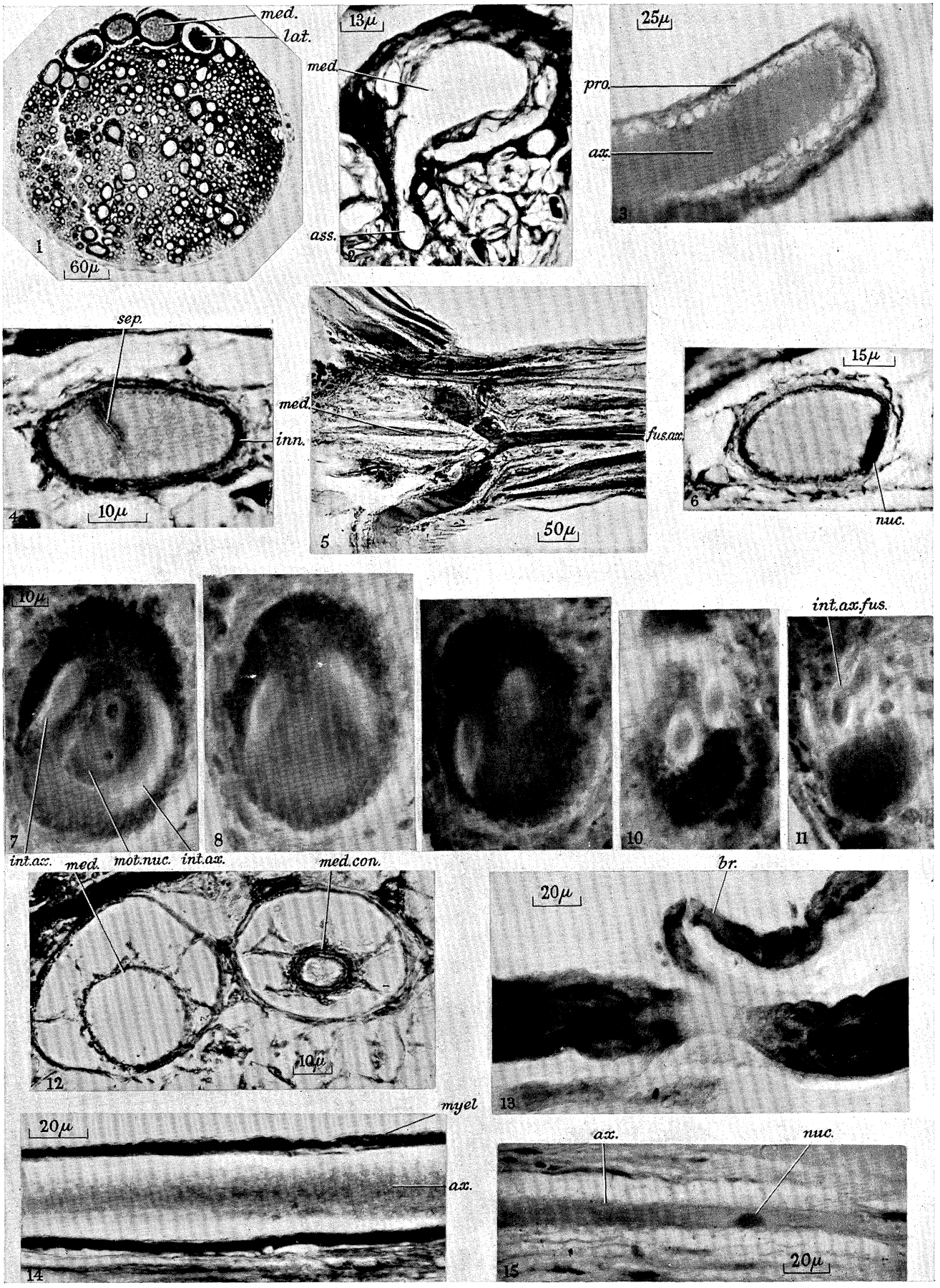
Figures 30, 31. Successive transverse sections of a median fibre showing a septum across the axoplasm. In figure 30 this seems completely to divide the axoplasm, but in figure 31 it can be seen to be incomplete. Picric acid fixation, Mallory-azan stain.

Figures 32–36. Transverse sections showing the relations between the two fibres at a lateral giant-fibre synapse. Picric acid fixation, Mallory-azan stain.

Figures 37–43. Transverse sections showing the relations between the two fibres at a lateral giant-fibre synapse. Osmium tetroxide fixation, safranin stain. Figures 40 and 41 are photographs of the same section taken with the objective focused on different planes.

Abbreviations used in the Plates

ass.	small axon associated with the median fibre
ax.	axon
ax.proc.	axonic processes at the synapse
br.	branch of the myelinated fibre, arising at the node
fus.ax.	axon formed by fusion of the motor fibres
inn.	'inner sheath'
int.ax.	intracellular axon
int.ax.fus.	fusion of the intracellular axons to form the motor fibre
lat.	lateral giant fibre
med.	median giant fibre
med.con.	median fibre showing the axonal constriction and thickened inner sheath
mot.	motor giant fibre
mot.nuc.	nucleus of the motor neuron
myel.	myelin sheath
myel.gap	gap in the myelin sheath at the synapse
n.med.	normal median giant fibre
nuc.	nucleus between axon and myelin layer
*op.med.*1	median fibre degenerated on the operated side
*op.med.*2	median fibre reappearing on the operated side
*op.med.*3	median fibre of normal appearance
pro.	protein network of the myelin sheath
sep.	incomplete septum in median fibre axon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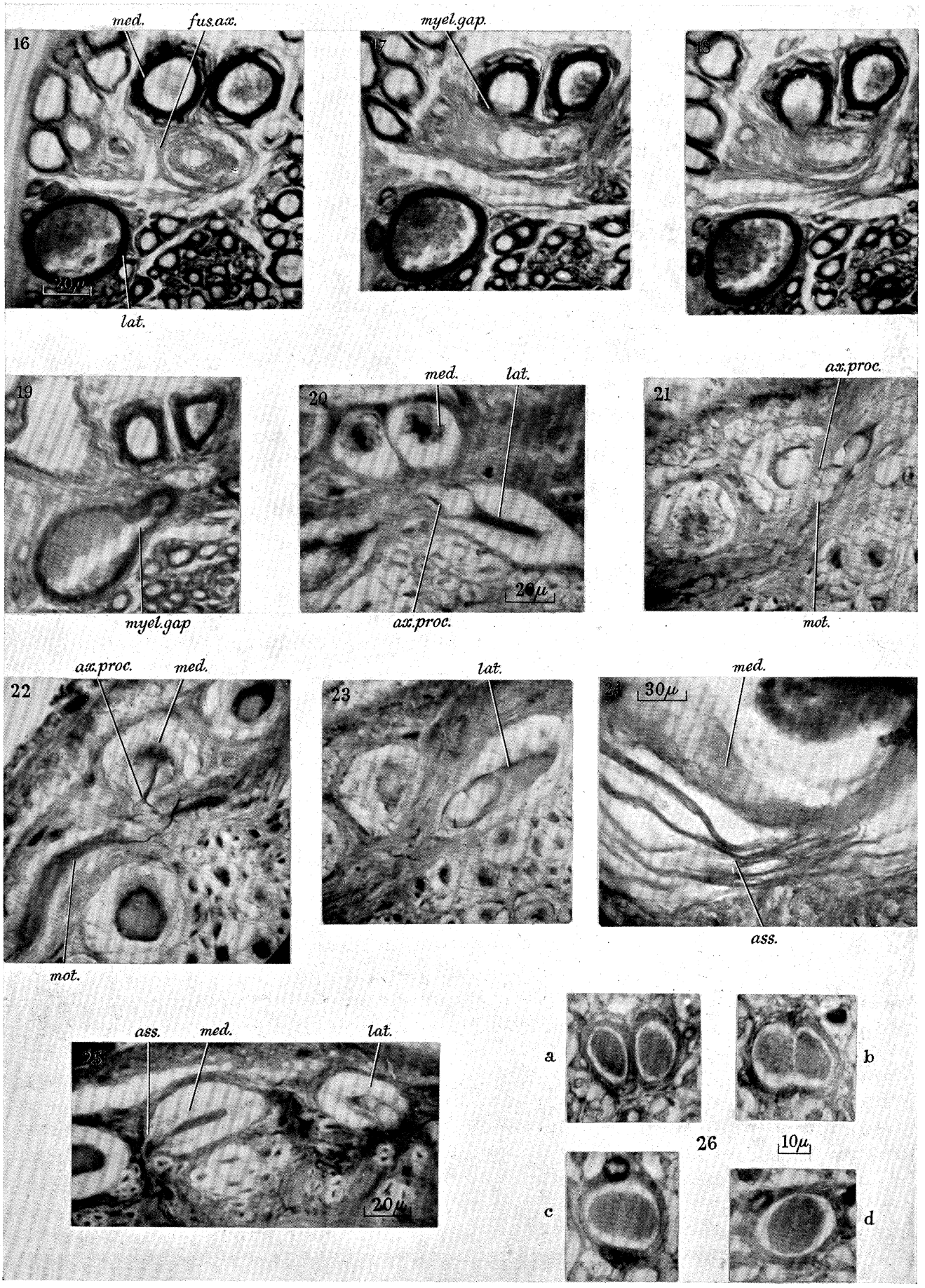
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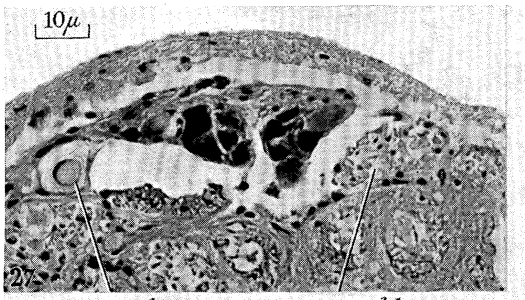
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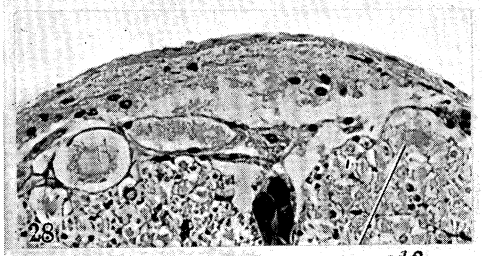
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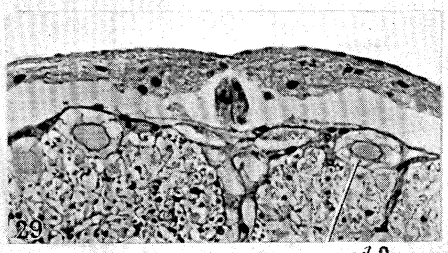
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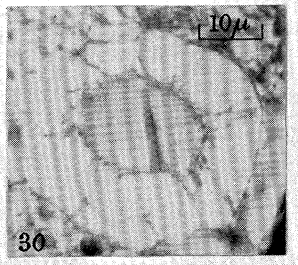
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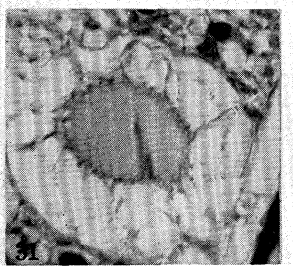
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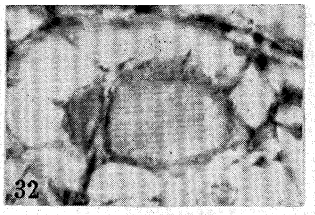
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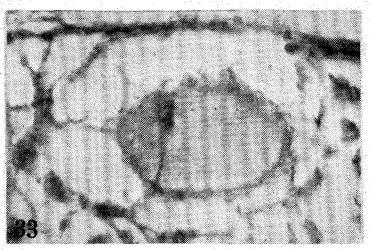
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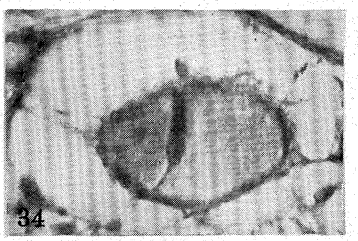
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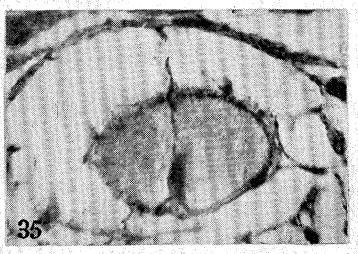
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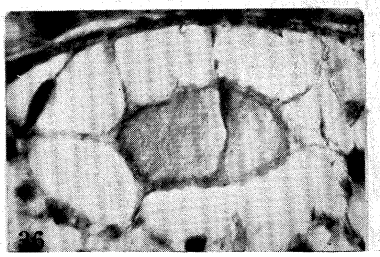
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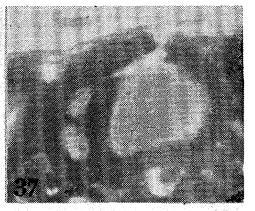
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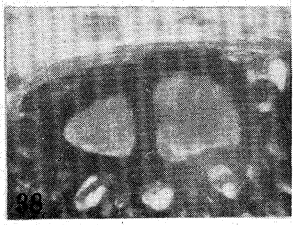
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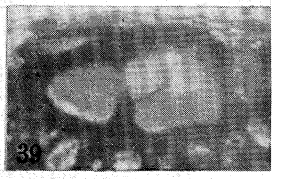
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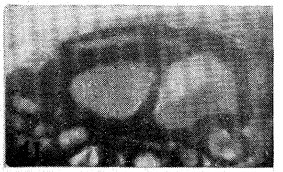
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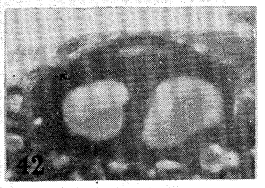
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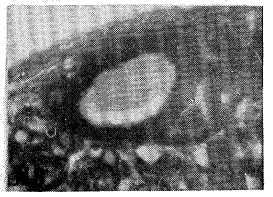
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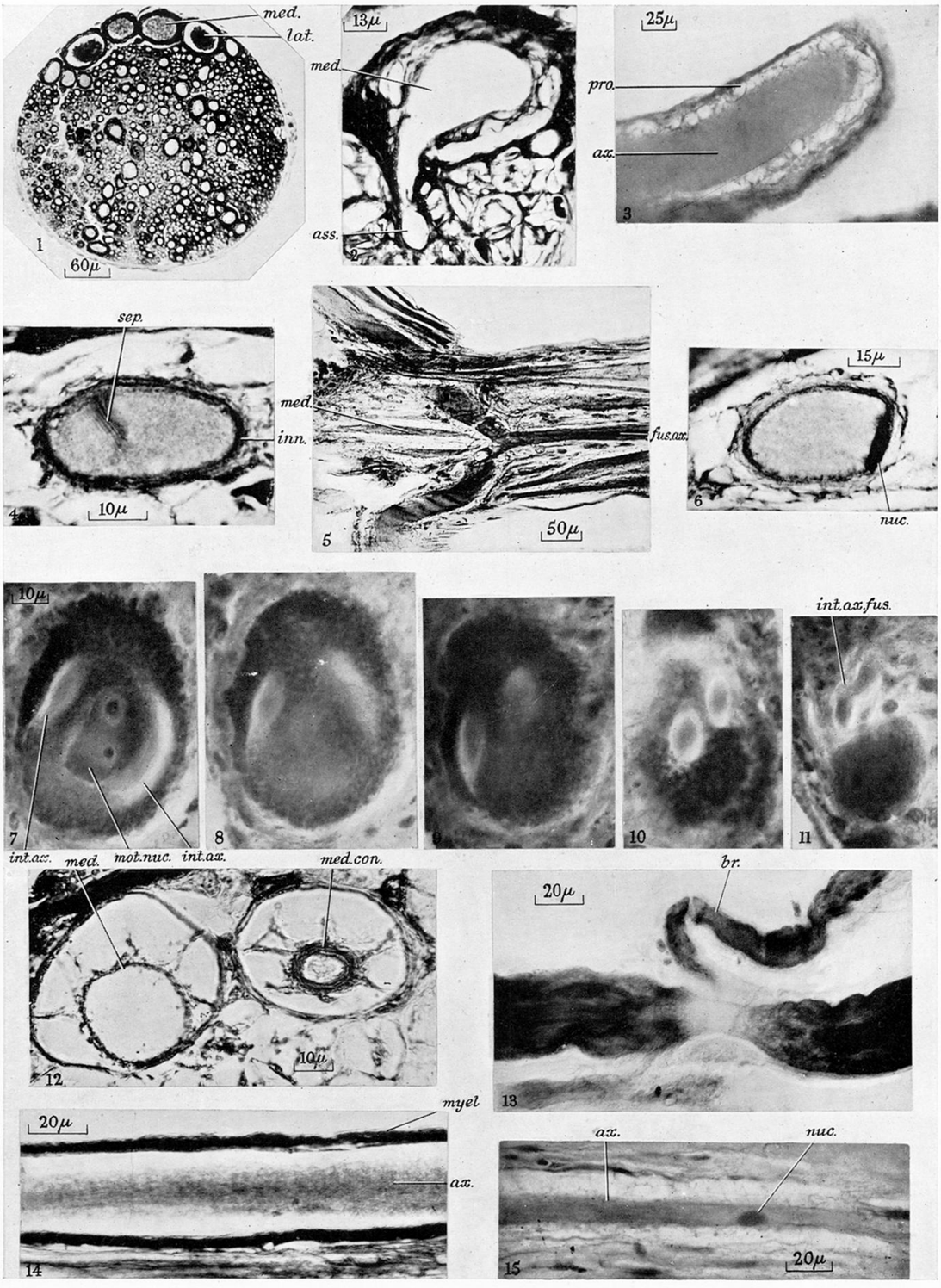


Plate 22

Figure 1. Transverse section of the central nervous system between two ganglia in the abdomen. Osmium tetroxide fixation.

Figure 2. Transverse section of median giant fibre in abdominal cord, showing an association between its axon and that of a smaller fibre. Fixation formaldehyde-sea water: material mordanted with potassium dichromate for a myelin sheath stain, but this has not been successful.

Figure 3. Longitudinal section of motor giant fibre immediately after leaving the central nervous system in the thoracic region. This fibre at this point is larger than any within the central nervous system. Picric acid fixation, celloidin embedding, stain haematoxylin and eosin.

Figure 4. Transverse section of median giant fibre in a thoracic ganglion, showing an incomplete septum. The septum has a fibrillary appearance. Picric acid fixation, stain haematoxylin and eosin.

Figure 5. Horizontal longitudinal section of the cord in the abdomen showing the division of the median fused axon into the two motor giant fibres, and the path of these out of the cord. Picric acid fixation, Bodian stain.

Figure 6. Transverse section of median fibre showing a nucleus between myelin and axon and the relations of the 'inner sheath'. Picric acid fixation, Mallory-azan stain.

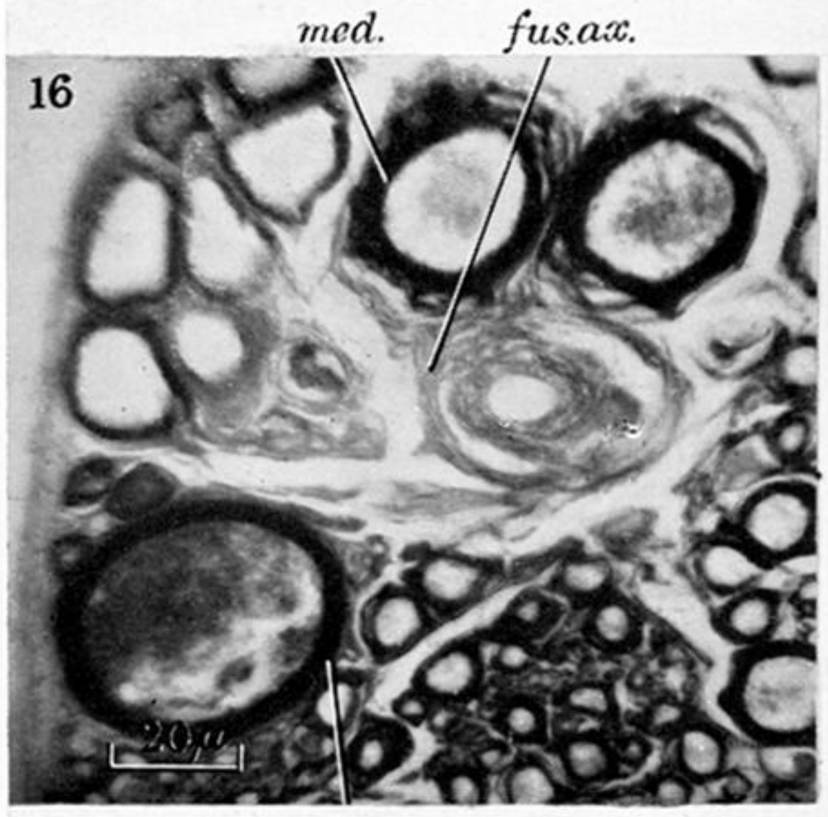
Figures 7–11. Successive transverse sections showing the origin of a motor fibre from a double axon in its large cell body. Figure 11 shows the two axons of origin about to fuse to form the motor fibre. Picric acid fixation, Bodian stain.

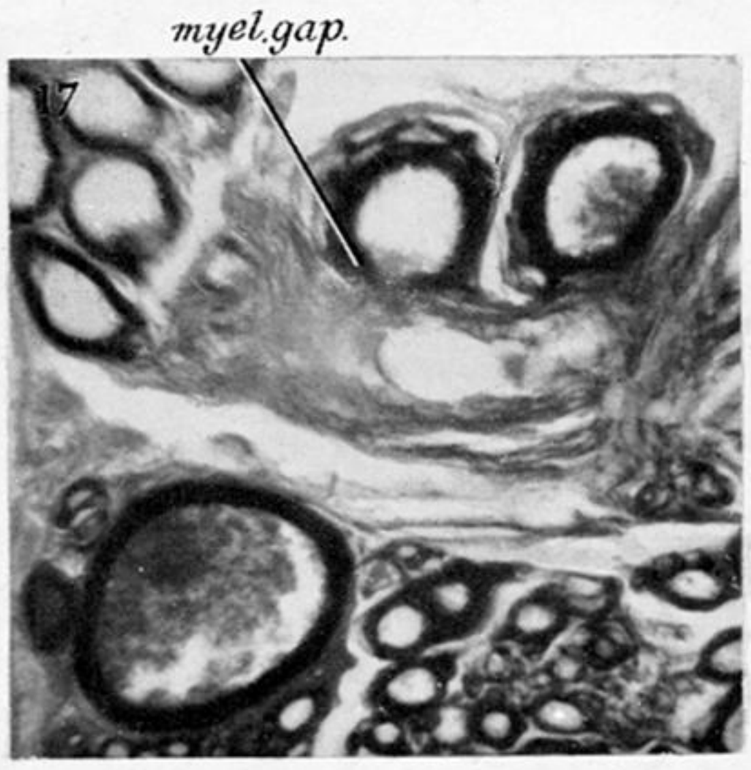
Figure 12. Transverse section of two median fibres in an abdominal ganglion. One of the fibres is of normal appearance; the other shows the segmental constriction of the axon with thickening of the inner sheath. Picric acid fixation; Mallory-azan stain.

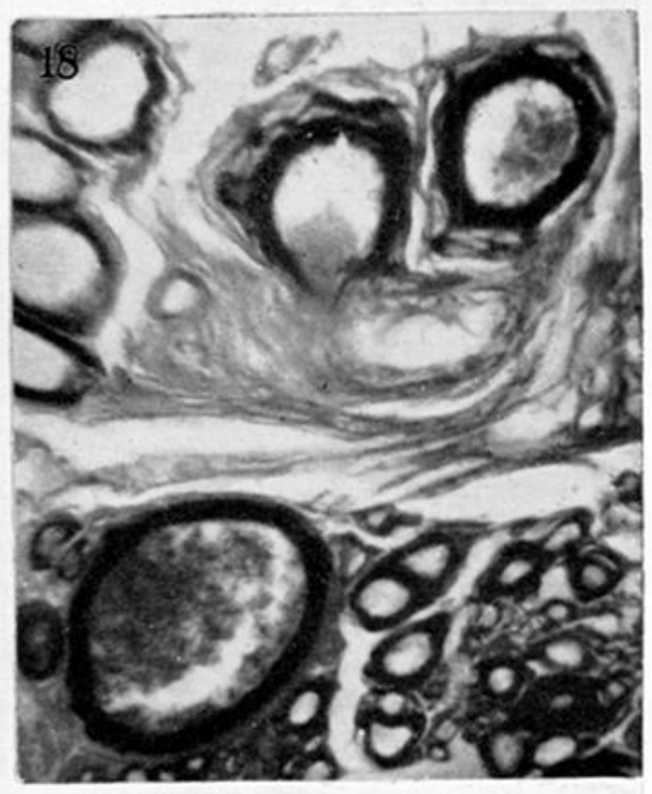
Figure 13. Whole mount of single teased fibre showing a Ranvier node, from the chela of the largest thoracic appendage. Fixation osmium tetroxide; preparation mounted in glycerin.

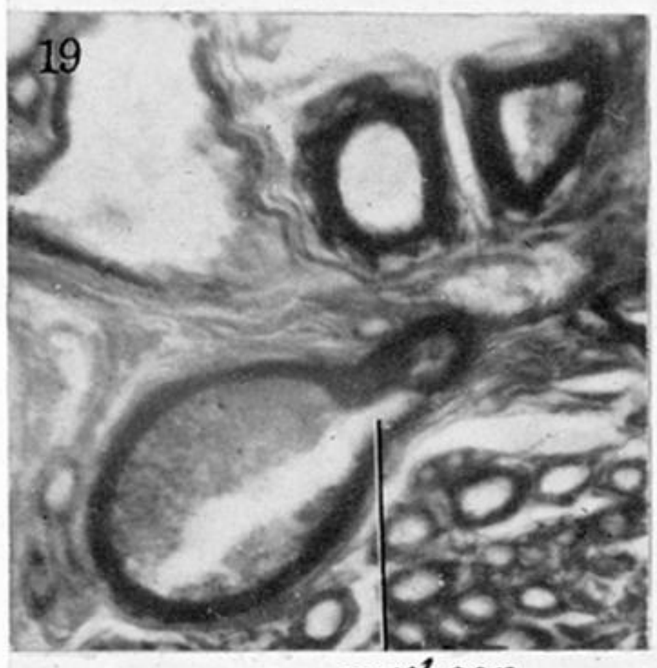
Figure 14. Longitudinal section of large fibre from the central nervous system, showing axonic shrinkage after osmium fixation. Fixation picro-osmic mixture.

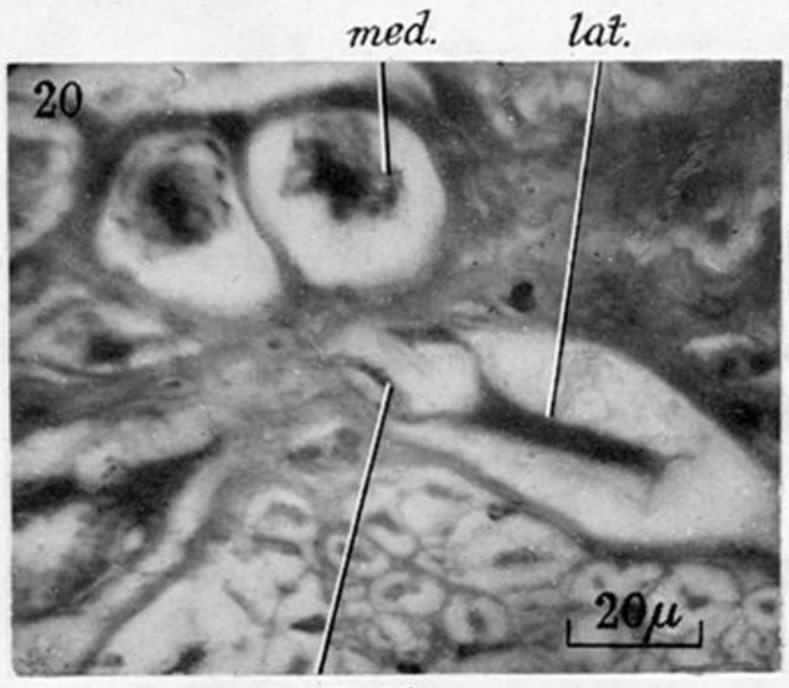
Figure 15. Longitudinal section of large fibre from the central nervous system showing the relation of the inner nucleus to the axon. Picric acid fixation, celloidin embedding, haematoxylin and eosin stain.

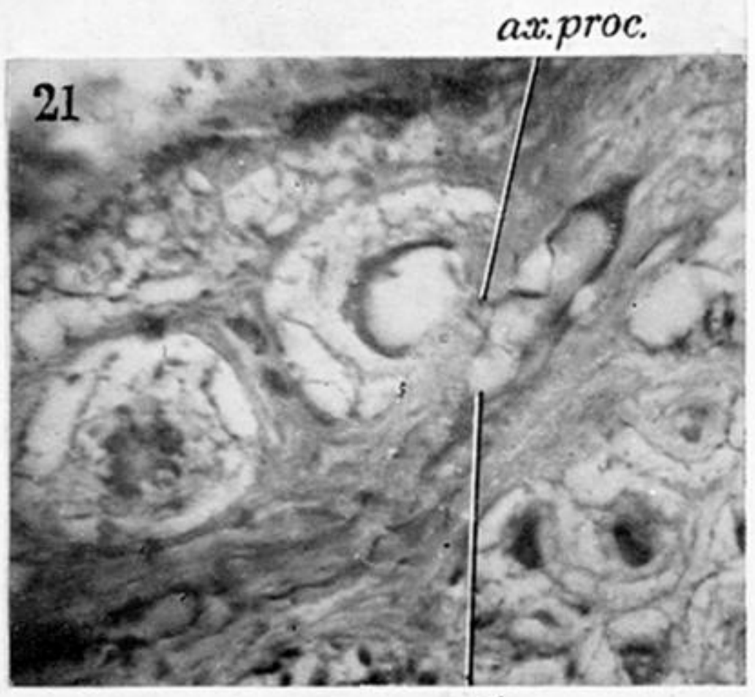


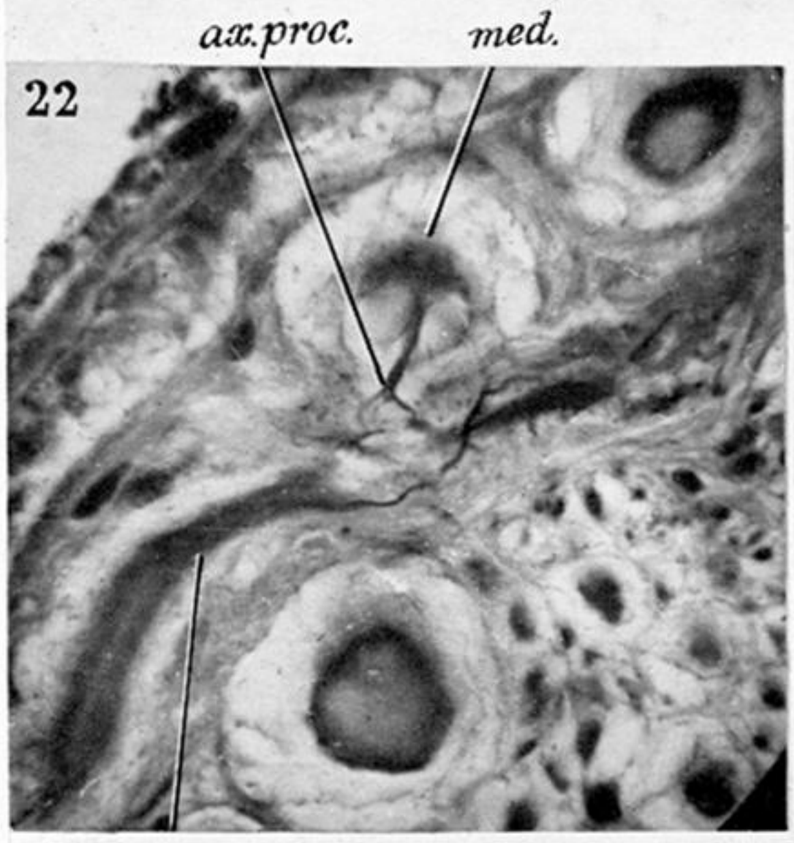


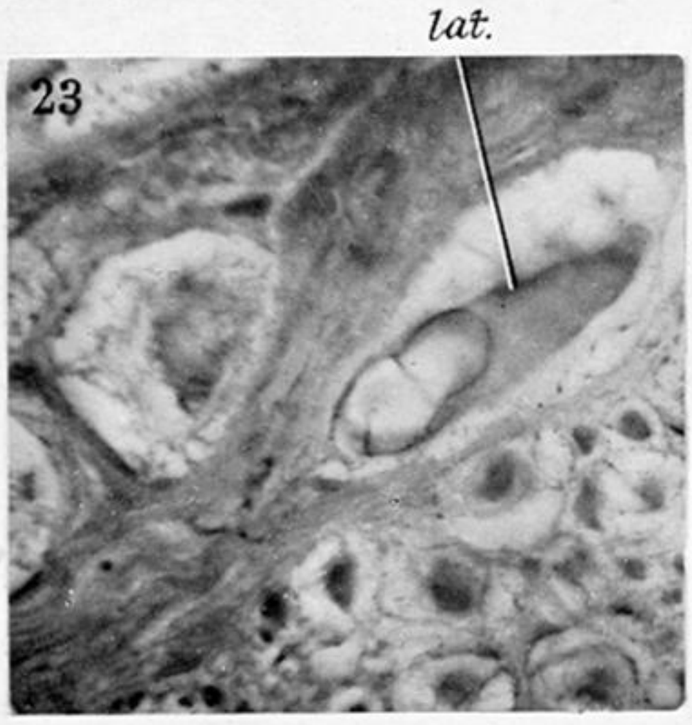


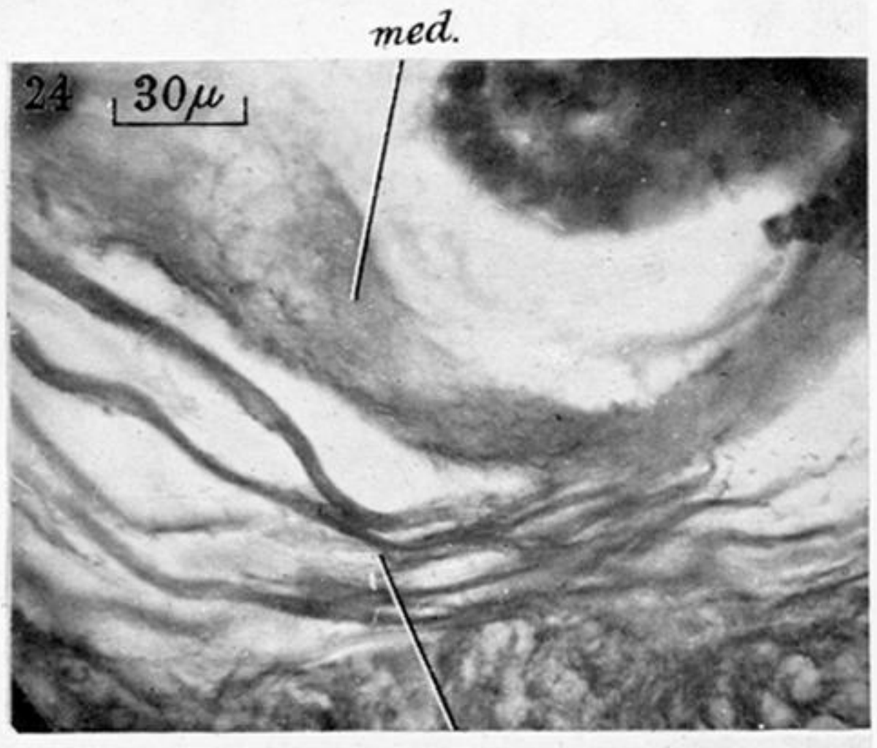












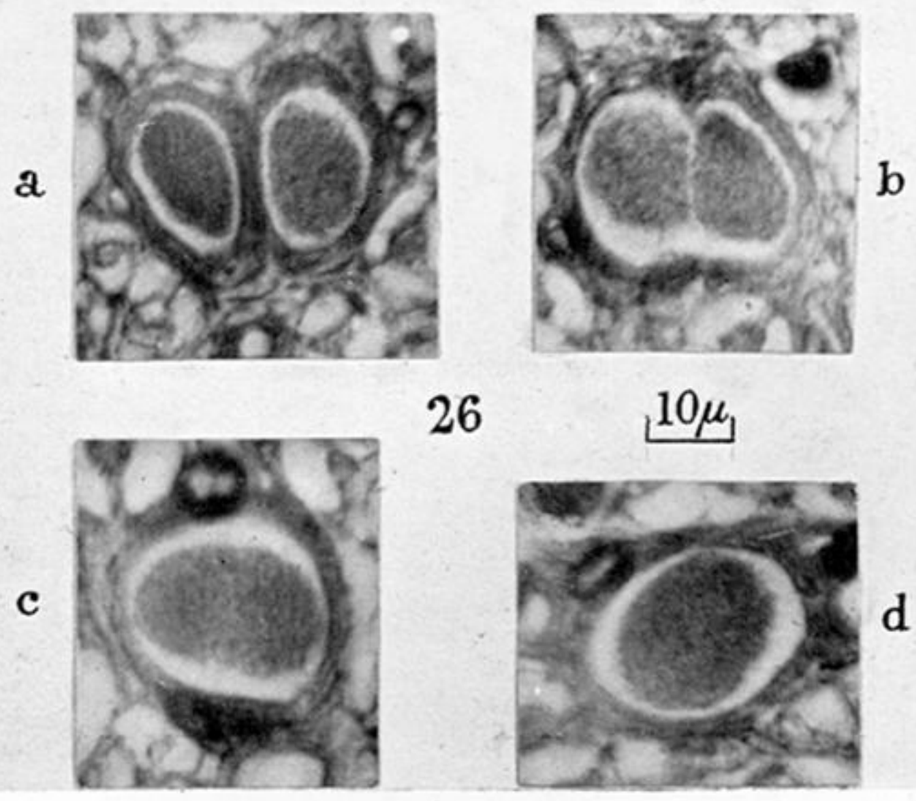


PLATE 23

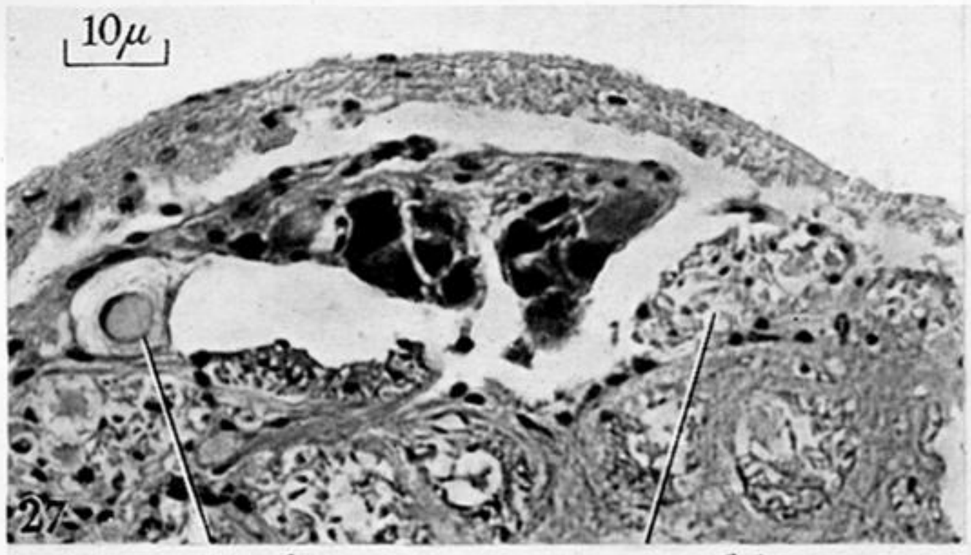
FIGURES 16–19. Successive sections, 8μ in thickness, showing the synaptic relations between the median and lateral fibres and the motor fibres in an osmium tetroxide preparation. All are to the same scale.

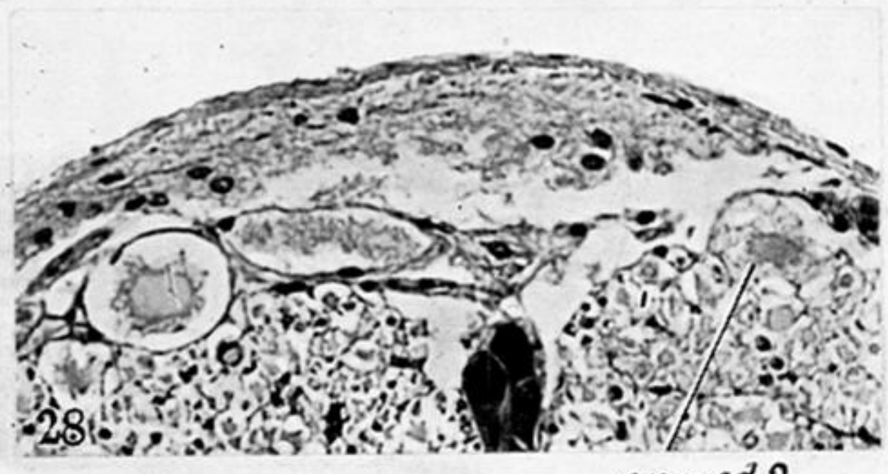
FIGURES 20–23. Successive sections, comparable with figures 16–19 showing the synaptic relations between the median and lateral fibres and the motor fibres in material fixed in picric acid and stained by Bodian's method. All are to the same scale.

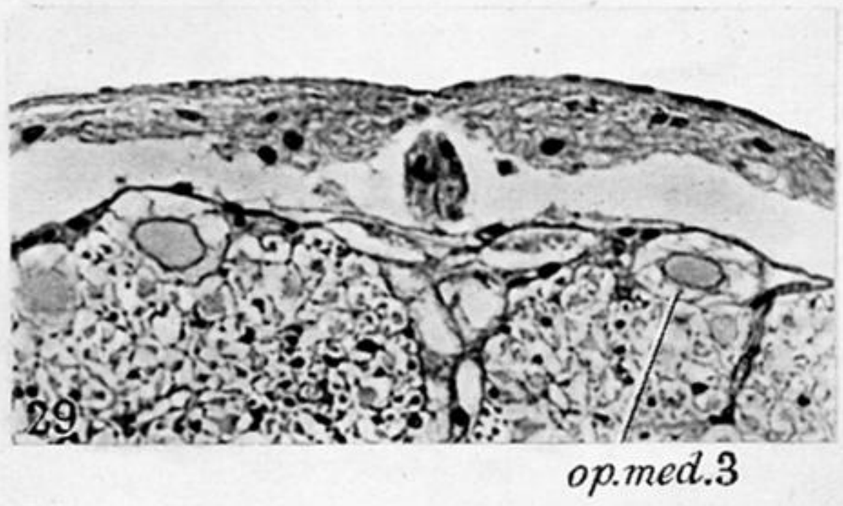
FIGURE 24. Longitudinal section of median fibre in a ganglion showing its relations with many smaller axons. Picric acid fixation, celloidin embedding, haematoxylin and eosin stain.

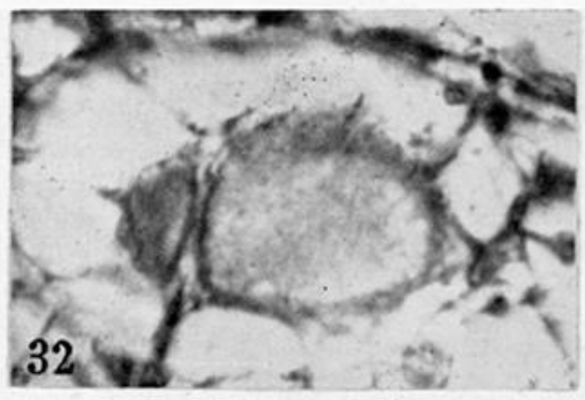
FIGURE 25. Transverse section of abdominal ganglion showing median fibre associated with a small axon, and the first appearance of the lateral fibre of the segment. Picric acid fixation, Bodian stain.

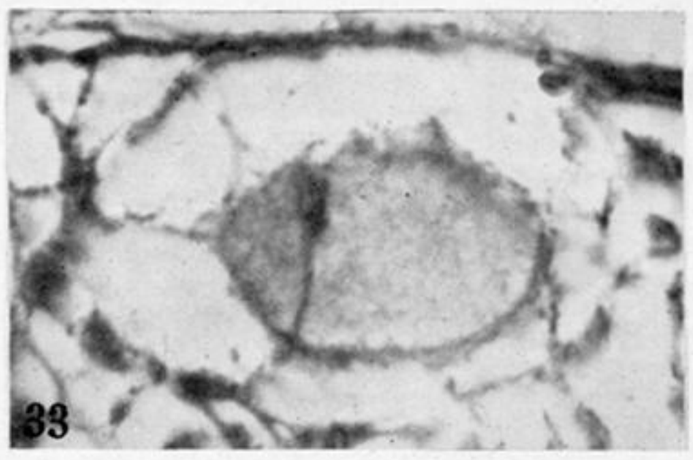
FIGURE 26 *a–d*. Transverse sections showing the successive stages in the fusion of the two motor axons to form the median fused axon. Picric acid fixation, haematoxylin and eosin.

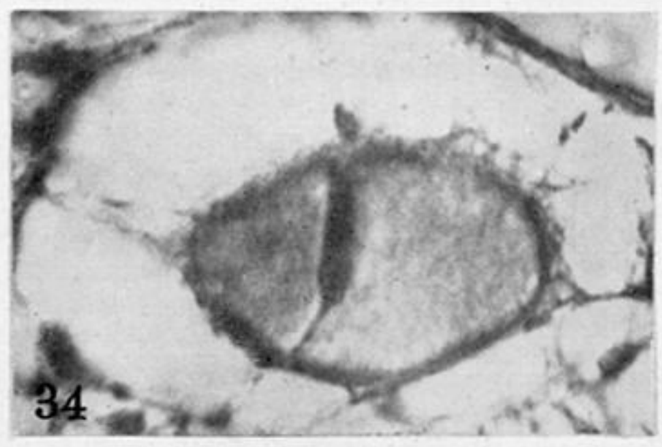


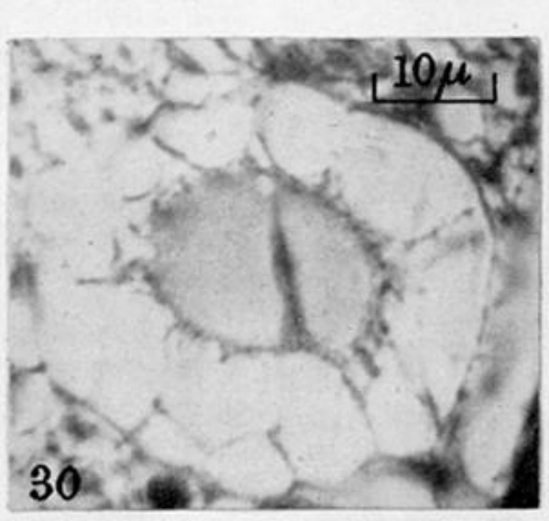


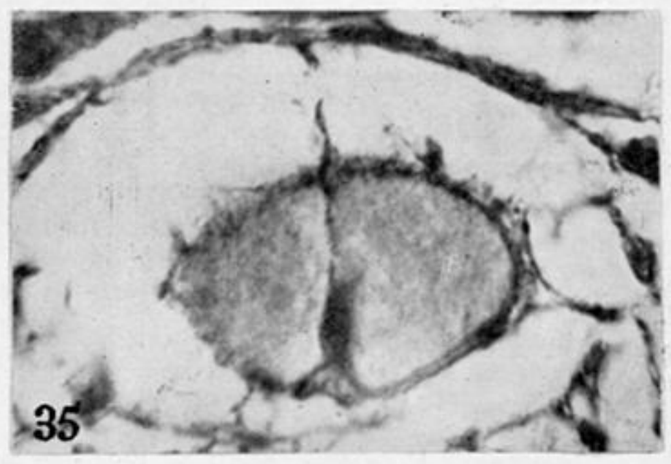


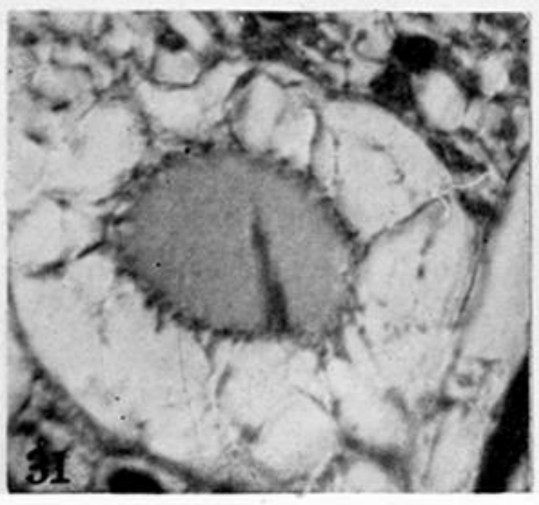


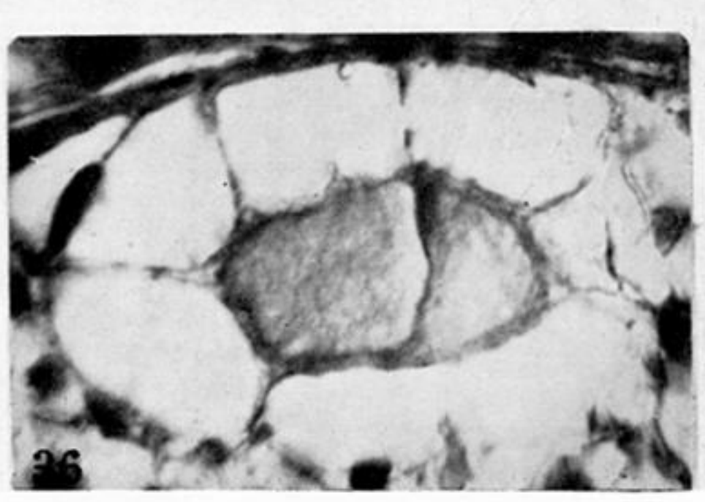


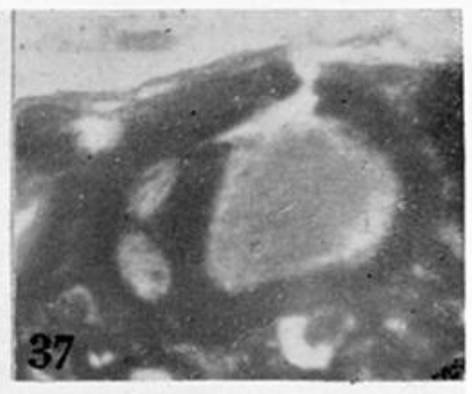


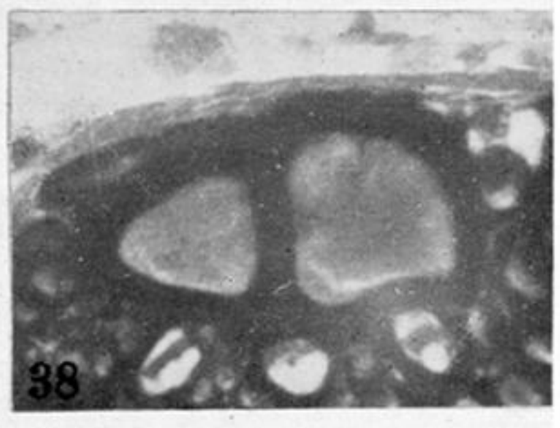


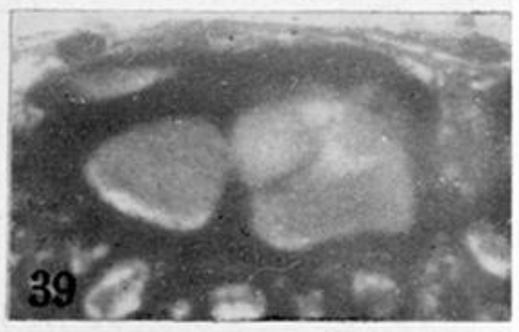


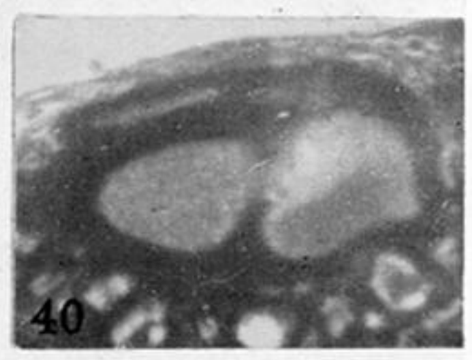


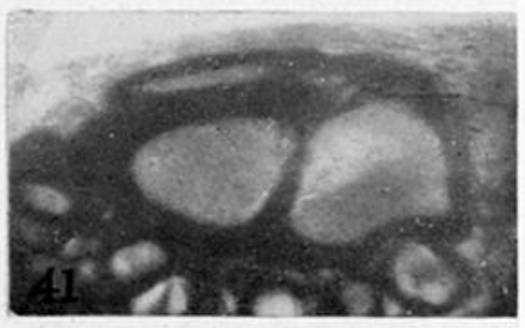


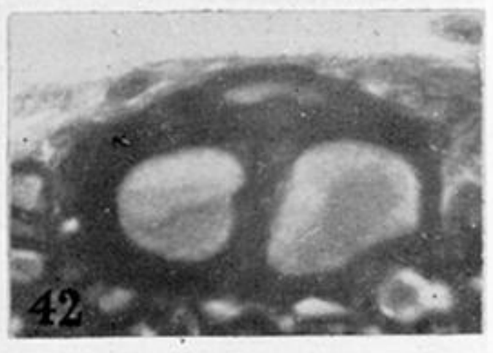












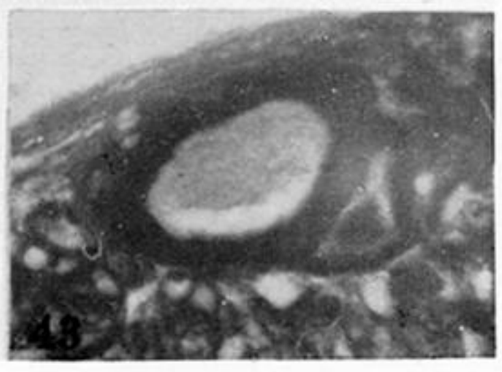


Plate 24

Figures 27–29. Successive transverse sections of the first thoracic ganglion of the nerve cord in an animal in which one circumoesophageal connective had been transected 12 days previously. The most anterior section, figure 27, shows no trace of the median fibre on the operated side, though that on the other side is normal. Figure 28 is posterior to figure 27 and shows the reappearance of the median fibre on the operated side. Figure 29 is the most posterior section, and shows that in the posterior region of the first thoracic ganglion the median fibre on the operated side is present and normal in appearance. All to the same scale. Picric acid fixation, haematoxylin and eosin.

Figures 30, 31. Successive transverse sections of a median fibre showing a septum across the axoplasm. In figure 30 this seems completely to divide the axoplasm, but in figure 31 it can be seen to be incomplete. Picric acid fixation, Mallory-azan stain.

Figures 32–36. Transverse sections showing the relations between the two fibres at a lateral giant-fibre synapse. Picric acid fixation, Mallory-azan stain.

Figures 37–43. Transverse sections showing the relations between the two fibres at a lateral giant-fibre synapse. Osmium tetroxide fixation, safranin stain. Figures 40 and 41 are photographs of the same section taken with the objective focused on different planes.